

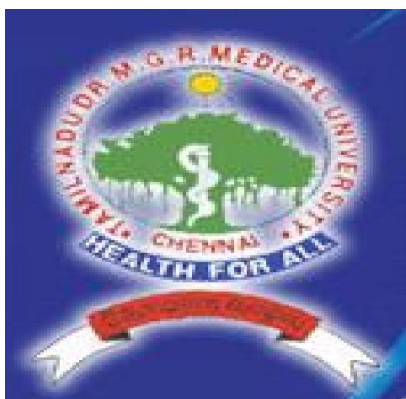
# **SAFETY AND PHARMACOLOGICAL PROFILE OF *INDHUPPU BHAVANAI***

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Chennai – 47**

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# INTRODUCTION

## INTRODUCTION

The Siddha system dates back to 5000 B.C profounded by Saint *Agathiyar* and his clan numbering 18 such Siddhars. This system was an amalgam of Tamil literature, culture, tradition, health and many such living forms of 64 types.

The Department of Gunapadam in National Institute of Siddha, Chennai is functioning from 30/9/2004 and many such research works had been carried out successfully..

The current research was on *Indhuppu bhavanai* to evaluate the safety and pharmacological activities in animal models.

The drug *Indhuppu bhavanai* was indicated for *Pitha Gunmam* (Hyperacidity) which was selected from the Siddha literature "*Kadukkai vallaaraiin thani maanbu*" third edition-1992, pg.no: 84, authored by *Hakkim P. Mohammed Abdulla sayub*.

All the ingredients were identified and authenticated by the experts and the test drug was prepared by the given procedure.

Review of literature in various categories were carried out. Siddha aspect, botanical aspect and mineralogical aspect disclosed about the drug and the disease, which strongly supports that it possesses anti- ulcer (Hyperacidity), anti-inflammatory and analgesic activities, for that purpose it has been selected for this study

The drug was subjected to analysis such as physicochemical, phytochemical, chemical and also instrumental analysis which provided the key ingredients present in the drug thus it accounts the efficacy of the drug

Preclinical evaluation of acute and Repeated dose 28 day oral toxicity study was carried out in K.K College of Pharmacy, Gerugambakkam and Repeated dose 90 day oral toxicity study of the drug was carried out in the Animal house, NIS, Chennai.

Wistar albino rats of either sex of weight 150-200 gm were used for toxicity and pharmacological studies. The animals were kept under standard conditions 12:12(day/night cycles) at room temperature in polypropylene cages. The animals were fed on standard pelleted diet and potable water in polypropylene bottles *ad libitum*. The animals were housed for one week prior to the experiments to acclimate to animal house conditions.

In acute toxicity study, various dose levels of *Indhuppu bhavanai* 5, 50, 300 and 2000 mg/kg b.w. was administered to female wistar albino rats which showed no abnormalities in external observation and necropsy examination and all the vital organs were normal.

In Repeated dose 28 day oral toxicity study and Repeated dose 90 day oral toxicity studies, various doses level of *Indhuppu bhavanai* at 900mg/kg and 1800 mg/kg was administered orally, did not show any significant changes in hematological parameters and histopathological slides of various organs.

In Pharmacological studies, Anti-ulcer (Hyperacidity), anti-inflammatory and analgesic activities of the drug was carried out in wistar albino rats and mice as per OECD guideline in K.K College of Pharmacy, Gerugambakkam revealed that the drug *Indhuppu bhavanai* exhibited significant anti-ulcer, anti-inflammatory and analgesic activities.

The above studies showed that the drug *Indhuppu bhavanai* was safe in animal studies and may be carried for clinical trial.

# AIM AND OBJECTIVES

## 2. AIM AND OBJECTIVES

### AIM

The aim of the study was to evaluate the Safety and Pharmacological profile of the test drug “*INDHUPPU BHAVANAI*” in animal models.

### OBJECTIVE

The following methodology was adopted to evaluate the Safety and Pharmacological activities of the test drug.

- Review of various information (Siddha and modern) relevant to the study.
- Preparation of the drug as per classical Siddha literature.
- Analytical study of the prepared drug
  - Physicochemical and phytochemical analysis
  - Chemical analysis to evaluate acidic and basic radicals.
  - Elemental analysis
  - Analysis of Particle size
- Screening the toxicity in animal models.
  - Acute oral toxicity study (OECD – 423 Guideline)
  - Repeated dose 28 day oral toxicity (OECD – 407 Guideline)
  - Repeated dose 90 day oral toxicity (OECD – 408 Guideline)
- Evaluation of pharmacological activities in animal models.
  - Anti -ulcer (Aspirin induced ulcer model)
  - Anti -inflammatory (Cotton pellet induced granuloma method)
  - Analgesic (Eddy’s hot plate method)



# MATERIALS AND METHODS

### 3. MATERIALS AND METHODS

#### STANDARD OPERATIVE PROCEDURE OF *INDHUPPU BHAVANAI*

##### Drug selection

The drug *Indhuppu Bhavanai*, a herbo-mineral formulation was taken as a trial drug from Siddha literature *Kadukkai Vallaraiin Thani Maanbu*, third edition-1992, pg.no: 84 ,Author:*Hakkim P. Mohammed Abdulla sayub*

The Ingredients of *Indhuppu Bhavanai* are, <sup>(3)</sup>

- |  |                   |
|--|-------------------|
| 1. <i>Purified Indhuppu</i> ( <i>Sodium chloride impura</i> )        | – 5 palam (175 g) |
| 2. <i>Vallarai leaf juice</i> ( <i>Centella asiatica</i> )           | } Required volume |
| 3. <i>Lemon pulp juice</i> ( <i>Citrus limon</i> )                   |                   |
| 4. <i>Purified fresh Ginger juice</i> ( <i>Zingiber officinale</i> ) |                   |
| 5. <i>Nellikai pulp juice</i> ( <i>Phyllanthus emblica</i> )         |                   |

##### Collection of the Plant materials

All plant materials were freshly collected from Tambaram sanatorium, Tamilnadu. *Indhuppu* was procured from a well reputed country shop in Parrys, Chennai. *Indhuppu* was purified and the medicine was prepared in the Gunapadam laboratory of National Institute of Siddha.

##### Identification and Authentication of the drug

Mineral drug was authenticated by the Chemist in Central Research Institute of Siddha, Arumbakkam, Chennai.

The Herbal drugs were Identified and authenticated by the Botanist, National Institute of Siddha.

## **Bhavanai<sup>(1)</sup>**

Bhavana was a process in which powders were soaked in various fluids, such as the expressed juice of herbs, decoctions etc., and then dried. For this purpose the quantity of juice added to the powder should be sufficient to cover it. The mixture is then allowed to dry in a shaded place. This process is repeated twice or thrice or as many times, as in necessary.

## **Purification process**

### ***Indhuppu*<sup>(2)</sup>**

*Indhuppu* was dissolved in vinegar and kept for 3 days and dried in sunlight.

## **Preparation of the drug<sup>(3)</sup>**

- *Indhuppu* was placed in *Kalvam* and powdered. *Vallarai* juice was poured until *Indhuppu* was immersed.
- Then it was grinded slowly till it loses moisture content and becomes dry and the same process was repeated for 7 times.
- Then the above mentioned process was carried out with the following juices respectively,

- Lemon juice

- Ginger juice

- Goose berry juice

Finally powder was dried & preserved.

## **Labelling**

Name of the preparation : *Indhuppu Bhavanai*

Date of preparation : 15/6/2015

Dose : 1 gm bd

Adjuvant/Vehicle : Honey

Indications : *Pitha gunmam* (hyperacidity), *Vaanthi* (Vomiting), *Vayitru eraichal* (Gastric irritation), *Seriyaamai* (Indigestion), *Vaayu muthaliya irappai sampanthamana noigal* (Gastro intestinal disorders) *theerum*.

Date of expiry : 100 years from the date of manufacture

### **INDHUPPU BHAVANAI**



## INGREDIENTS OF *INDHUPPU BHAVANAI*

### *Indhuppu*

**Before purification**



**After purification**



***VALLARAI* (*Centella asiatica*)**



***INJI* (*Zingiber officinalae*)**



***ELUMICHAI* (*Citrus limon*)**



***Nellikai* (*Phyllanthus emblica*)**



# REVIEW OF LITERATURE

# GUNAPADAM REVIEW

## GUNAPADAM REVIEW

### இந்துப்பு - INDHUPPU

#### வேறு பெயர்கள்

- சைந்தவம், சிந்தாரம், சந்திரனுப்பு, மதிகூர்மை, மதியுப்பு, மிந்தாச்சொல்.
- இது ஒரு மண்பூத சரக்காகும்.
- இதில் பச்சைக் கற்பூரம் மடியும், பூரம் முப்பாகும்.

#### செய்கை

மலகாரிச் செய்கை

#### பொதுகுணம்

அட்ட குன்ம மந்தம் அசிக்கரஞ்சூர் சீதபித்தந்

துட்டவையம் நாடிப்புண் தோடங்கள்- கெட்டமலக்

கட்டுவிட விந்தையக் காமியநோய் வன்கரப்பான்

விட்டுவிட விந்துப்பை விள்.

சென்னிக்கண்ணா பற்றூர் செவிகவுள்கண் டம்பகநோய்

சந்நியா சங்காசந் தாகமிரைப்புன்னிரத்த

மூலஞ் சிலந்திநளி மூடிகநஞ் சூதை வலி

சூலஞ் சிதையுமிந்தாற் சொல்.

#### பொருள்

எண்வித குன்மம், அலசம், அசிக்கரம், நரம்புக்கிரந்தி, கடுவன், மலபந்தம், விடம், திரிதோடம், கபாதிக்கம், தலை, விழி, நா, தந்தமூலம், தாது, கன்னம், கண்டம், யோனி இவ்விடத்து நோய்கள், இரத்த மூலம், சுவாசம், சிலந்தி, தேள், எலி இவற்றின் விடங்கள், சூலை தீரும்.



### மருத்துவ பயன்கள்

- இந்துப்பை தேகத்தில் சுளுக்கு வந்தால் பற்று போடலாம்.
- இந்துப்பை வலியுடன் கூடிய வீக்கங்களுக்கு சூடாக்கி ஒற்றடம் இடலாம்.

### இந்துப்பு சேரும் பிற மருந்துகள்

- 1) இந்துப்புச் சூரணம்
- 2) சங்கத் திராவகம்
- 3) அற்புத மாத்திரை
- 4) சித்தாதி எண்ணெய்
- 5) நவ உப்பு மெழுகு
- 6) குன்மகுடோரி மெழுகு
- 7) பஞ்சலவண பற்பம்
- 8) நாயுருவி உப்பாதிக் குழம்பு

## வல்லாரை - VALLARAI

வேறு பெயர்கள்<sup>(5)</sup> : சண்டகி, பிண்டகி, யோசனவல்லி

**Botanical name** : *Centella asiatica* (Linn.)

**English name** : Indian Pennywort

**Family** : Apiaceae

பயன்படும் உறுப்பு

பூண்டு

### Organoleptic characters

சுவை : துவர்ப்பு, கைப்பு, இனிப்பு

தன்மை : தட்பம்

பிரிவு : இனிப்பு

செய்கை

உடற்றேற்றி

உரமாக்ககி

பொதுகுணம்

அக்கர நோய் மாறும் அகலும் வயிற்றிழிவு

தக்கவிரத் தக்கடுப்புத் தானேகும் - பக்கத்தில்

எல்லாரை யுமருந்தென் றேயுரைத்து நன்மனையுள்

வல்லாரை யைவளர்த்து வை.

(தேரையர் குணவாகடம்)

பொருள்

வாய்ப்புண், கழிச்சல், குருதிக் கழிச்சலால் பிறக்கும் வயிற்றுக் கடுப்பு நீங்கும்.

### மருத்துவ பயன்கள்

- இலையை அரைத்துக் கட்ட, யானைக்கால். விரை வீக்கம், வாயு வீக்கம், அடிபட்டத் தசை சிதைந்துண்டான வீக்கம் போகும்.
- குழந்தைகளுக்குண்டாகும் சீதக்கழிச்சல், குருதிக் கழிச்சல், வயிற்றுக் கடுப்பு முதலியவைகளுக்கு அதன் இலையை அரைத்துக் கொப்பூழ் மீது பற்றிட அவைகள் நீங்கும்
- இலையுடன் வெந்தயமும் சேர்த்து குடிநீரிட்டு 1, 2 சங்கு குழந்தைகளுக்குப் புகட்ட, சுரம், வயிற்றுக் கோளாறுகள் போம்.
- வல்லாரை இலை பொடியில் 130-300 மி.கி எடுத்து பசுவின் பாலில் கலந்து கொடுக்க, ஞாபக சக்தி உண்டாகும். மூளையும் பலப்படும்.
- வல்லாரை இலை பொடியை புண்கள் மீது தூவி வர புண்கள் எளிதில் ஆறும்.

### வல்லாரை சேரும் பிற மருந்துகள்

- 1) வல்லாரை நெய்
- 2) அமிர்தசஞ்சீவி லேகியம்
- 3) வல்லாரை மணப்பாகு
- 4) திரிபலை மாத்திரை
- 5) மிளகு பக்குவம்
- 6) திரிகடுகு மாத்திரை

**எலுமிச்சை - *Citrus limon***

வேறு பெயர்கள்<sup>(5)</sup> : சம்பீரம்

**English name** : Lime

**Botanical name** : *Citrus limon* (Linn) Burm.f.

**Family** : Rutaceae

பயன்படும் உறுப்பு

பழம், பழரசம்

**Organoleptic characters**

இலை, காய், பழம்

சுவை : புளிப்பு

தன்மை : வெப்பம்

பிரிவு : கார்ப்பு

செய்கை

பழம் : குளிர்ச்சியுண்டாக்கி

பொதுகுணம்

பழம்

தாகம் குநகநோய் தாழாச் சிலிபதநோய்

வேகங்கொள் உன்மாதம் வீறுபித்தம்- மாகண்ணோய்

குன்னனோய் வாந்தியும்போங் கட்டுவா தித்தொழிலில்

முன்னெலுமிச் சங்கனியை வாழ்த்து.

(அகத்தியர் குணவாகடம்)

பொருள் :

மயக்கம், வாந்தி, வாய்க்குமட்டல், நீர்வேட்கை, வெறி, கண்ணோய், காதுவலி போகும்.

### சம்பீரம்

மந்திரிக்கு மந்திரியாய் மன்னனுக்கு மன்னனெனத்

தந்திரிக்கு மித்திரன்போற் சாருமே- முந்தவரு

கம்பீர மாய்ச்சரக்கின் கெண்ணியமாய் வாகடர்க்குச்

சம்பீர மாமெலுமிச் சை.

(தேரன் காப்பியம்)

### பொருள்

எலுமிச்சம் பழம் மந்திரி எனக்கூறும் தீக்குற்றத்தைத் தணிக்கும், தந்திரியாகிய ஐயத்திற்கு அன்பன்போலிருந்து அனல் வளர்க்கும்.

(தே.வெண்பா)

### மருத்துவ பயன்கள்

- இப்பழரசத்தோடு சர்க்கரை கூட்டி நீர் சேர்த்துப் பெருக்கி நீர்விடாய்க்கு அருந்தலாம்.
- எலுமிச்சம்பழத்தை கற்பமுறையாய்ப் பத்தியத்துடன் ஆறுமாதங் கொள்ள பிடிப்பு, பெருவயிறு, பக்ககுலை, முடம், மனச்சோர்வு, மயக்கம் தீரும்.
- பவள பற்பத்தை இப்பழச் சாற்றுடன் சேர்த்து சீதக் கழிச்சல், பெருங் கழிச்சல், அதிசார கழிச்சல் இவைகளுக்குக் கொடுக்கலாம்.
- சீரகத்தை தேன் விட்டுப் பொன்வறுவலாக வறுத்து, இதன் பழரசத்தைச் சேர்த்து நீர்விட்டுக் காய்ச்சிக் கொடுக்க, உடனே வாந்தியும் கழிச்சலும் நிற்கும்.

### எலுமிச்சை சேரும் பிற மருந்துகள்

- 1) சிந்தாதி லேகியம்
- 2) பூர்ணாதி லேகியம்
- 3) தாம்பிர பற்பம்
- 4) இலவண பற்பம்
- 5) கலிங்காதி எண்ணெய்
- 6) இஞ்சி லேகியம்

## இஞ்சி - *Zingiber officinale*

வேறு பெயர்கள்<sup>(5)</sup> : அல்லம், ஆர்த்தரகம், ஆத்திரகம், இலாக்கொட்டை, நறுமருப்பு மதில்

**English name** : Green ginger fresh root

**Botanical name** : *Zingiber officinale*

**Family** : Zingiberaceae

பயன்படும் உறுப்பு

கிழங்கு

### Organoleptic characters

சுவை : கார்ப்பு

தன்மை : வெப்பம்

பிரிவு : கார்ப்பு

### செய்கை

- அகட்டுவாய்வகற்றி
- பசித்தீத்தூண்டி
- உமிழ்நீர்ப்பெருக்கி
- செரிப்புண்டாக்கி

### பொதுகுணம்

இஞ்சிக் கிழங்குக் கிருமல்ஜயம் ஓக்காளம்

வஞ்சிக்குஞ் சன்னிசரம் வன்பேதி- விஞ்சுகின்ற

குலையறும் வாதம்போந் தூண்டாத தீபனமாம்

வேலையுறுங் கண்ணாய்- விளம்பு

(அகத்தியர் குணவாகடம்)

இஞ்சியின் குணமே தென்றி யல்புட னுரைக்கக் கேளீர்

அஞ்சிடுஞ் சன்னியெல்லா மகன்றிடும் பித்த தோடம்

நெஞ்சினி லிருமற் கோழை நெகிழ்ந்திடும் கபங்கள் தன்னை

மிஞ்சினி வருமோ வென்று விளம்பிடும் தேவ நூலே (௭௫)

### பொருள்

இஞ்சியினால் அழல்குற்றம், வளிகுலை, முக்குற்ற நோய்கள், செரியாக்கழிச்சல் போகும். பசியுண்டாகும்.

### மருத்துவ பயன்கள்

- அகட்டைப் பற்றிய வயிற்று வலி, வயிற்றுப் பொருமல் வாந்தி, குடல் நோய், முதலிய நோய்களுக்கு இஞ்சியை தேனூறல் செய்து கொடுக்கலாம்.
- செரியாக் கழிச்சலுக்கு இஞ்சிச் சாற்றை, தொப்புளைச் சுற்றித் தடவவும்.
- இஞ்சிச் சாறும், வெங்காயச் சாறும் வகைக்கு ஒரு தோலா வீதம் எடுத்துக் கலந்துக் கொடுக்க வாந்தி, ஓக்காளம் தீரும்.
- இஞ்சியை பாலில் அரைத்துக் கழற்சியளவு 18 மி.லிட்டர் பாலில் கலந்து காலை மாலை கொடுத்துவர இருமல், இளைப்பு, குன்மம், மயக்கம், அழல் வாய்வு தீரும்.

### இஞ்சிசேரும் பிற மருந்துகள்

- 1) நாரங்காதி லேகியம்
- 2) இஞ்சி லேகியம்
- 3) சிந்தாதி லேகியம்
- 4) பூர்ணாதி லேகியம்
- 5) புளியாரை நெய்
- 6) இஞ்சி சூரணம்

## நெல்லி - *Phyllanthus emblica*

வேறு பெயர்கள்<sup>(5)</sup> : ஆமலகம், ஆலகம், ஆம்பல், ஆமரிகம், தாத்தாரி, தாத்திரி, கோரங்கம், மிறுதுபலா, மீதுந்து

**Botanical name** : *Phyllanthus emblica*. Linn

**English name** : Indian gooseberry

**Family** : Euphorbiaceae

பயன்படும் உறுப்பு

காய்

### Organoleptic characters

சுவை : புளிப்பு, துவர்ப்பு, இனிப்பு

தன்மை : தட்பம்

பிரிவு : இனிப்பு

செய்கை

பழம் : குளிர்ச்சியுண்டாக்கி

பொதுகுணம்

பித்தமன லையம் பீநிசம்வாய் நீர் வாந்தி

மத்தமலக் காடும் மயக்கமுமில்-ஒத்தவுரு

வில்லிக்கா யம்மருங்கா மென்னாட்கா லந்தேர்ந்தே

நெல்லிகா யம்மருந் துணீ.

(தேரையர் குணவாகடம்)



நெல்லிக்காய்க் குப்பித்தம் நீங்கு மதன்புளிப்பால்

செல்லுமே வாதமதிற் சேர்துவரால்- சொல்லுமையம்

ஒடுமிதைச் சித்தத்தில் உன்ன அனலுடனே

கூடுபிற மேகமும் போங் கூறு.

(தேரையர் குணவாகடம்)

**பொருள் :**

வாந்தி, மயக்கம், வாய்நீர்ச் சுரப்பு தீரும். அதன் புளிப்பால் துவர்ப்பால் ஐயமும் நீங்கும்.

**மருத்துவ பயன்கள்**

- நெல்லிக்காயைத் துவையல் செய்து சாப்பிட, சுவையின்மை, வாந்தி இவைகளைப் போக்கும்.
- நெல்லிவற்றலைக் குடிநீர் செய்து கொடுக்க மயக்கம், தாகம், ஓக்காளம் நீங்கும்.
- இலைக் கொழுந்தை அரைத்து மோரில் கலந்து சீதக்கழிச்சலுக்குக் கொடுக்கலாம்.

**நெல்லிக்காய்சேரும் பிற மருந்துகள்**

- 1) சரபங்க வில்வாதி லேகியம்
- 2) நாரத்தை லேகியம்
- 3) இஞ்சி லேகியம்
- 4) நெல்லிக்காய் லேகியம்
- 5) கரிசாலை லேகியம்

# BOTANICAL REVIEW

## 4.2 BOTANICAL ASPECT

### ***VALLARAI - CENTELLA ASIATICA***

**Synonym<sup>(6)</sup>** : *Hydrocotyle asiatica* Linn

**Family** : **Apiaceae**

#### **Vernacular names**

**Tamil** : *Vallarai , Chandaki*

**English** : Indian pennywort

**Hindi** : Khulakudi

#### **Parts used**

Leaf

#### **Organoleptic characters**

**Taste** : Astringent, bitter, sweet

**Nature** : Neutral

**Division** : Sweet

#### **Action**

Alternative, Tonic, Diuretic, Stimulant, Emmenagogue.

#### **Chemical constituents**

**Leaves** : Essential oil, fatty oil, sitosterols, tannin and resinous substances

**Plant** : Triterpenoid asiaticoside – A and B

#### **Major Chemical constituents<sup>(8)</sup>**

Madecassoside, Asiaticoside, Asiatic acid, Brahmic acid

## **Minor chemical constituents**

Asiaticoside B, Brahminoside, Brahmoside, Centelloside, Indcentelloside, Thankuniside, Isothankuniside, Brahmic acid, Isobrahmic acid, Betulic acid, Centic acid, Centoic acid and flavanoid glycosides.

## **RESEARCH ARTICLE RELATED TO THE STUDY**

### **Acute and sub acute toxicity study<sup>(9)</sup>**

Acute toxicity study of *Centella asiatica* revealed that the drug at the dose of 10gm/kg did not cause any toxic signs and death within the observation period of 14 days.

Sub chronic toxicity study of ECa<sub>233</sub> at the doses of 10, 100, 1000 mg/kg/day showed no difference with regards to body weight, food consumption and health in comparison to the control group except that female rats receiving 1000mg/kg/day of ECa<sub>233</sub> had significantly higher white blood cell counts than the control group.

### **Pharmacological activities**

#### **Anti ulcer activity<sup>(10)</sup>**

The research article revealed that the *Centella asiatica* extract of dose 100mg/kg, 200mg/kg, 400mg/kg exhibited protection of gastric mucosal layer. Histopathological studies reveals reduction or absence of edema and leucocytes infiltration of submucosal layer. It also possess analgesic and anti-inflammatory activity<sup>(34)</sup>

#### **Medicinal uses<sup>(7)</sup>**

- The extract from the plant promotes epithelialisation and a very early decrease in the wound surface area.
- It cures haemorrhagic diseases and heart trouble, efficacious in skin affections, anorexia, subduces deranged pitta.
- The leaves of *Centellia asiatica* is fried in castor oil and applied over swellings in elephantiasis and scrotal swelling.

### ***Elumichai - Citrus limon***

**Synonym<sup>(6)</sup>** : *Citrus medica* L.

**Family** : Rutaceae

#### **Vernacular names**

Tamil : *Elumichchai, arasakani, Kesari*

Hindi : Nimbu

English : Acid lime, limon

#### **Parts used**

Fruit

#### **Organoleptic Characters**

##### **Unripe fruit, fruit**

Taste : Sour

Nature : Hot

Division : Acrid

#### **Action**

Lemon juice : Refrigerent, antiscorbutic action

fruit : Refrigerant

#### **Chemical constituents**

Lemons are a rich source of vitamin C. Lemons contain numerous phytochemicals, including polyphenols and terpenes. Abscissic acid, Gibberellic acid, Limonin, Linoleic acid, Ascorbic acid, Carotenoids, Pantothenic acid, pectin and vitamin B<sub>1</sub>. Fresh lemon juice contains minerals such as Zinc, Selenium, Manganese, Iron, Sodium, Chloride, Calcium. It also contains Vitamin A, Niacin, Vitamin C, Vitamin E and Vitamin K. As with other citrus fruits, they have significant concentrations of citric acid (about 47 g/l in juice).

## RESEARCH ARTICLES RELATED TO THE STUDY

### Anti-ulcer activity<sup>(33)</sup>

The research article revealed that the healthy male albino wistar rats of weight 200-250 g were pretreated with the extract of *Citrus medica* at the dose level of 250 mg kg<sup>-1</sup> and 500mg/kg-1 showed a significant reduction in ulcer formation. Histopathological sections showed significant decrease in mucosal ulceration, inflammatory mucosal changes and submucosal edema compared to ethanol treated group and ranitidine group.

It is concluded that the fruits of *Citrus medica* possesses significant anti-ulcer activity against ethanol-induced ulcers in rats and the anti-ulcer activity could be due to the presence of flavonoids as these compounds have well documented anti-ulcer activity.

### Analgesic activity<sup>(11)</sup>

The research article revealed that the fruit decoction of *Citrus medica* Linn. were found to be effective in a dose level of 4ml/kg which inhibited the pain produced by the hot plate. Thus it is concluded that this study validates the traditional use of *Citrus medica* Linn. as an analgesic.

### Medicinal uses<sup>(7)</sup>

- The fruit is efficacious in the treatment of vomiting and gastric stimulant.
- The ripe fruit is highly appetizing.
- It is highly recommended in rheumatism affections such as plerodynia, sciatica, lumbago, pain in the hip joints.

## ***Zinziber officinale - Inji***

### **Vernacular names<sup>(6)</sup>**

Tamil	:	<i>Inchi, allam, ilaakottai, narumaruoou mathil</i>
English	:	Ginger
Hindi	:	Adrakh

### **Parts used**

Bulbous root or rhizome

### **Organoleptic characters**

Taste	:	Acrid
Nature	:	Hot
Division	:	Acrid

### **Action**

Stimulant, Carminative, Stomachic, Sialagogue, Digestive, Rubefacient

### **Chemical constituents<sup>(8)</sup>**

#### **Major:**

Gingerol,  $\alpha$  – Zingiberene,  $\beta$ - sesquiphellandrene and  $\alpha$  - curcumene, Lipids, Proteins and Starch.

#### **Minor:**

- Gingerdiols,
- Gingerdiacetates,
- Gingerdiones,
- Diterpenes, 6- gingersulfonic acid,
- Gingerglycolipids A, B and C.

## RESEARCH ARTICLES RELATED TO THE STUDY

### Toxicity study<sup>(12)</sup>

The research article revealed that the aqueous extract of ginger rhizome at the dose of 2g/kg b.w. for 14 days indicated that there were no lethal effect upto 1g/kg b.w. and no adverse effect on the major organs.

### Pharmacological activity<sup>(13)</sup>

The research article revealed that the aqueous extract of ginger root extract of dose 200mg/kg, 400mg/kg when administered to rats, the percentage inhibition of gastric ulcer was 40.91%, 57.58% respectively.

This shows that the ginger root extract significantly inhibited the gastric damage induced by indomethacin and its efficacy as a gastro protective agent was comparable to that of Omeprazole.

Another study reveals that the active components in ginger root could have an antisecretory activity and cytoprotection by increasing mucus wall thickness.

Ginger and its constituents act as digestive aids: possess antiulcer<sup>(18-24)</sup>, Cholagogic<sup>(25)</sup> and antiemetic<sup>(26-28)</sup> properties.

### Medicinal uses<sup>(7)</sup>

- Ginger decoction is given for colic pain, tympanites and vomiting.
- It is aromatic and beneficial in dropsy, dyspepsia, digestive and stomachic.
- Ginger juice rubbed on and around navel cures all kinds of diarrhoea.



***Emblica officinalis* - Nellikai**

**Synonyms<sup>(6)</sup>** : *Phyllanthus emblica* Linn.; *Cicca emblica* Kurz

**Common name** : Emblic myrobalan, Indian Goose berry.

**Family** : Euphorbiaceae

**Vernacular names**

Tamil : *Nelli*

English : Gooseberry

Hindi : Amlika

**Parts used**

Fruit

**Organoleptic characters**

Taste : Astringent, sweet, sour

Nature : Neutral

Division : Sweet

**Action**

Fruit : Diuretic, refrigerant, laxative

**Chemical Constituents<sup>(8)</sup>**

**Fruit**

- Trigalloylglucose, terchebin, covilagin, ellagic acid, phyllembic acid
- Vitamin C, Gallic acid, Ellagic acid, Phyllembic acid, emblicol
- Phyllantidine, Phyllantine, Pectin, Minerals

## RESEARCH ARTICLES RELATED TO THE STUDY

### Acute toxicity study<sup>(14)</sup>

The acute and chronic toxicity study revealed that the hydroalcoholic extract of amla (HAE) was prepared and was administered at the dose of 250, 500 and 1000 mg/kg body orally does not cause any mortality up to 48 hr. Hence the LD<sub>50</sub> of *Emblica officinalis* is more than 1000 mg/kg and there was no untoward effect and no gross observational effects were observed at any dose of *Emblica officinalis*.

### Pharmacological activity<sup>(16)</sup>

The research article revealed that the aqueous extract of *Phyllanthus emblica* (PE-AqE 47) when administered to rats inhibited the H.pylori growth while its antisecretory, anti-ulcer and gastroprotective effects may be due to its potent antioxidant characteristics. Its significant ulcer protective and healing effects and this might be due to its effects both on offensive and defensive mucosal factors.

### Medicinal uses<sup>(7)</sup>

- It is effective in the treatment of peptic ulcer<sup>(25-27)</sup> and dyspepsia<sup>(28)</sup>.
- The leaves of *Phyllanthus emblica* is boiled in water and goggled for mouth ulcers. Decoction of goose berry will remove excessive thirst, giddiness and vomiting sensation.
- Fruits are good liver tonic. The fruit juice is useful in indigestion, jaundice, anaemia, heart complaints. It is a rich source of vitamin C.

# MINERALOGICAL REVIEW

### 4.3 MINERALOGICAL ASPECT

#### Sodium Chloride Impura – Rock salt

##### Vernacular names

**Tamil** : *Indhuppu*

**Eng** : Rock salt

**Hindi** : Sendhalon

**Halite** commonly known as **rock salt**, is the mineral form of sodium chloride (NaCl). Halite forms isometric crystals.

##### Source

Found in nature in extensive beds mostly associated with clay and calcium sulphate.

##### Characters

- It is found in small white crystalline grains or transparent cubes.
- It is brownish white externally and white internally.
- It has a pure saline taste and burns with a yellow flame.

##### Physicochemical properties

- Category : Halide mineral
- Chemical formula : NaCl
- Crystal symmetry : Isometrical hexoctahedral
- Molar mass : 58.433g/mol
- Crystal system : Cubic
- Luster : Vitreous
- Streak : White
- Optical properties : Isotropic
- Refractive index : 1.544
- Solubility : Water soluble
- Other characters : Salty flavour, Fluorescent

**Action**

- Carminative
- Stomachic
- Digestive

**Medicinal uses**

- It is given in dyspepsia and other abdominal disorders.
- It promotes the appetite and assists digestion and assimilation.
- It is made into a paste and applied in case of sprain
- Hot fomentation of the rock salt can be taken for curing the painful swellings.

ANALYTICAL STUDY OF  
*INDHUPPU BHAVANAI*

## 5. ANALYTICAL STUDY OF THE DRUG *INDHUPPU BHAVANAI*

Analytical study of the drug brings the validation to be used as a medicine by subjecting the drug to many analysis and determining its quality and effectiveness. Analytical study includes many studies such as its organoleptic properties, physico chemical properties and phytochemical properties and also to assess the active principles and elements present in the drug. Thus analytical study brings the efficacy and potency of the drug.

As per AYUSH protocol for analytical study, the following parameters were evaluated.

Analytical study of the drug includes:

➤ **Organoleptic characters**

- Colour
- Odour
- Taste
- Texture

➤ **Physicochemical analysis**

- Determination of Ash Values
- Physical characterization

➤ **Phytochemical analysis**

HPTLC and TLC finger print analysis

➤ **Chemical analysis**

Preliminary Basic and Acidic radical studies

➤ **Elemental analysis**

Inductively Coupled Plasma Optical Emissions Spectrometry (ICP- OES)

➤ **Analysis of particle size**

Scanned Electron Emission (SEM)

PHYSICO CHEMICAL  
ANALYSIS



## **5.1 Organoleptic characterization of *Indhuppu bhavanai***

The organoleptic characters of the sample drug were evaluated. 1gm of the test drug was taken and the following characters were seen.

Colour, Odour, Taste, Texture and other morphology were viewed by naked eye under sunlight. Then the result was noted.

### **Colour**

The medicine was taken into watch glasses and placed against white back ground in white tube light. It was observed for its colour by naked eye.

### **Odour**

The medicine was smelled individually. The time interval among two smelling was kept 2 minutes to nullify the effect of previous smelling.

The results of Organoleptic character were showed in table - 1

## **5.2 PHYSICOCHEMICAL ANALYSIS**

Physicochemical studies of the trial drug have been done according to the WHO guidelines

### **Physical properties of *Indhuppu bhavanai***

The physical properties of *Indhuppu bhavanai* was analyzed at Captain Srinivasamurti Reseach Institute for Ayurveda and Siddha Drug Development, Arumbakkam, Ch-106.

#### **1.Determination of pH:**

Five grams of *Indhuppu bhavanai* was weighed accurately and placed in clear 100 ml beaker. Then 50 ml of distilled water was added to it and dissolved well. After 30 minutes it was then applied in to pH meter at standard buffer solution of 4.0, 7.0, and 9.2. Repeated the test four times and average was recorded.

## **2.Loss on drying of the sample at 105°C -**

4g of test drug was weighed in a previously weighed 100ml beaker and heated in an oven at 105°C for 5hours. Cooled in a dessicator and weighed. Repeated the procedure till constant weight was obtained. The percentage loss in weight of the test drug was calculated by the following formula.

### **Calculation:**

$$\text{Percentage of loss on drying at } 105^{\circ}\text{C} = \frac{\text{Loss in weight of test drug}}{\text{Weight of test drug taken}} \times 100$$

## **3.Ash content**

### **Total ash content**

4g of test drug was weighed accurately in a previously ignited and tared silica dish. The material was evenly spread and ignited in a muffle furnace at 600°C until it became white indicating the absence of carbon. The dish was cooled in a dessicator and weighed. As carbon free ash cannot be obtained in this manner, the dish was cooled and the residue moistened with 2 sufficient quantity of water. Dried on a water bath and then ignited in the electric furnace to get the constant weight. Cooled the dish in a dessicator and then weighed. The percentage of total ash of air-dried materials was calculated as per the formula given below.

### **Calculation:**

$$\text{Percentage of total ash} = \frac{\text{Weight of the ash}}{\text{Weight of test drug taken}} \times 100$$

## **4.Acid-insoluble ash**

The total ash of the test drug was found out as described above. To the dish containing the total ash was added 45 ml of 1: 5 hydrochloric acid in three portions of 13 ml each time. Boiled gently for 5 minutes and filtered. Collected the insoluble matter on an ashless filter paper (Whatman No.41) and washed with distilled water until the residue was free from acid. Transfer the filter paper containing the insoluble mater to the original dish. Dried and ignited to the constant weight. Cooled the dish in a dessicator, and then weighed. Calculation was made by given formula.

**Calculation:**

$$\text{Percentage of acid-insoluble ash} = \frac{\text{Weight of the acid-insoluble residue}}{\text{Weight of test drug taken}} \times 100$$

**5. Water-soluble extractive of the test drug**

4 g of the test drug was weighed accurately in a glass stoppered flask. Added 100 ml of distilled water and shaken occasionally for 6 hours and then allowed to stand for 18 hours. Filtered rapidly taking care not to lose any solvent and pipetted out 25 ml of the filtrate in a pre weighed 100 ml beaker and evaporated to dryness on a water bath. Kept in an air oven at 105°C for 6 hours. Cooled in a dessicator and weighed. Repeated the experiment twice, and taken the average value. The percentage of water soluble extractive was calculated by the formula given below.

**Calculation:**

$$\text{Percentage of water soluble extract} = \frac{\text{Weight of the extract}}{\text{Weight of sample taken}} \times \frac{100}{25} \times 100$$

**6. Alcohol-soluble extractive of the sample**

4 g of the sample was weighed accurately in a glass stoppered flask. Added 100 ml of distilled alcohol (approximately 95%) and shaken occasionally for 6 hours and then allowed to stand for 18 hours. Filtered rapidly taking care not to lose any solvent and pipetted out 25 ml of the filtrate in a pre weighed 100 ml beaker and evaporated to dryness on a water bath. Kept in an air oven at 105°C for 6 hours and cooled in a dessicator and weighed. Repeated the experiment twice, and taken the average value. The percentage of alcohol soluble extractive was calculated by the formula given below.

**Calculation:**

$$\text{Percentage of alcohol soluble extract} = \frac{\text{Weight of the extract}}{\text{Weight of sample taken}} \times \frac{100}{25} \times 100$$

**RESULTS**

The results of physico chemical properties were represented in table 2.

# CHEMICAL ANALYSIS

### 5.3 CHEMICAL ANALYSIS OF *INDHUPPU BHAVANAI*

The chemical analysis of *Indhuppu Bhavanai* was carried out in Bio chemistry Lab, National Institute of Siddha.

S.No	EXPERIMENT	OBSERVATION	INFERENCE
1.	Physical Appearance of extract	Dark brown in colour	
2.	<b>Test for Silicate</b> a. A 500 mg of the sample was shaken well with distilled water.	Completely soluble	Absence of Silicate
3.	<b>Action of Heat:</b> A 500 mg of the sample was taken in a dry test tube and heated gently at first and then strong.	No White fumes evolved. No brown fumes evolved.	Absence of Carbonate Absence of Nitrate.
4.	<b>Flame Test:</b> A 500 mg of the sample was made into a paste with con. HCl in a watch glass and introduced into non-luminous part of the Bunsen flame.	No bluish green flame appeared	Absence of copper
5.	<b>Ash Test:</b> A filter paper was soaked into a mixture of extract and dil. cobalt nitrate solution and introduced into the Bunsen flame and ignited.	No yellow colour flame appeared	Absence of sodium

#### Preparation of Extract:

5 gm of *Indhuppu bhavanai* (IB) was taken in a 250 ml clean beaker and added with 50ml of distilled water. Then it is boiled well for about 10 minutes. Then it is cooled and filtered in a 100 ml volumetric flask and made up to 100 ml with distilled water. This preparation is used for the qualitative analysis of acidic/basic radicals and chemical constituents in it.

S. No	EXPERIMENT	OBSERVATION	INFERENCE
	<b>I. Test For Acid Radicals</b>		
1.	<b>Test For Sulphate:</b> a. 2 ml of the above prepared extract was taken in a test tube to this add ed 2 ml of 4% dil ammonium oxalate solution  b. 2 ml of the above prepared extract is added with 2 ml of diluted HCL is added until the effervescence ceases off. Then 2 m of Barium Chloride solution is added.	Cloudy appearance present  A white precipitate insoluble in con.HCL was obtained.	<b>Presence of Sulphate</b>  <b>Sulphate was confirmed</b>
2.	<b>Test For Chloride:</b> 2 ml of the above prepared extracts was added with 2 ml of dil- HCl is added until the effervescence ceases off.	Cloudy appearance present.	<b>Presence of Chloride</b>
3.	<b>Test For Phosphate:</b> 2 ml of the extract were treated with 2 ml of dil.ammonium molybdate solution and 2 ml of con.HNO <sub>3</sub>	No Cloudy yellow appearance formed	Absence of Phosphate
4.	<b>Test For Carbonate:</b> 2 ml of the extract was treated with 2 ml diluted magnesium sulphate solution	No cloudy appearance	Absence of carbonate
5.	<b>Test For Nitrate:</b> 1 gm of the extract was heated with copper turning and concentrated H <sub>2</sub> SO <sub>4</sub> and viewed the test tube vertically down.	No Brown gas was evolved	Absence of nitrate
6.	<b>Test For Sulphide:</b> 1 gm of the extract was treated with 2 ml of con. HCL	No rotten egg smelling gas evolved	Absence of sulphide
7.	<b>Test For Fluoride &amp; Oxalate:</b> 2 ml of extract was added with 2 ml of dil. Acetic acid and 2 ml dil.calcium chloride solution and heated.	No cloudy appearance.	Absence of fluoride and oxalate
8.	<b>Test For Nitrite:</b> 3 drops of the extract was placed on a filter paper, on that-2 drops of dil.acetic acid and 2 drops of dil.Benzidine solution is placed.	No characteristic changes	Absence of nitrite

9.	<b>Test For Borate:</b> 2 Pinches (50mg) of the extract was made into paste by using dil. sulphuric acid and alcohol (95%) and introduced into the blue flame.	Bluish green colour flame not appeared	Absence of borate
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S.No	EXPERIMENT	OBSERVATION	INFERENCE
<b>II. Test For Basic Radicals</b>			
1.	<b>Test For Lead:</b> 2ml of the extract was added with 2ml of dil. potassium iodine solution.	No Yellow precipitate appeared	Absence of lead
2.	<b>Test For Copper:</b> a. One pinch (25 mg) of extract was made into paste with con. HCl in a watch glass and introduced into the non-luminous part of the flame.	No blue colour precipitate appeared	Absence of copper
3.	<b>Test For Aluminium:</b> To the 2 ml of extract dil.sodium hydroxide was added in 5 drops to excess.	No characteristic changes	Absence of Aluminium.
4.	<b>Test For Iron:</b> a. To the 2 ml of extract add 2 ml of dil.ammonium solution b. To the 2ml of extract 2ml thiocyanate solution and 2ml of con HNO <sub>3</sub> is added	Mild Red colour appeared	<b>Presence of Iron</b>
5.	<b>Test For Zinc:</b> To 2 ml of the extract dil.sodium hydroxide solution was added in 5 drops to excess and dil.ammonium chloride is added.	No White precipitate was formed	Absence of Zinc
6.	<b>Test For Calcium:</b> 2 ml of the extract was added with 2 ml of 4% dil.ammonium oxalate solution	Cloudy appearance and white precipitate was obtained	<b>Presence of calcium</b>
7.	<b>Test For Magnesium:</b> To 2 ml of extract dil.sodium hydroxide solution was added in drops to excess.	No White precipitate was obtained	Absence of magnesium

8.	<b>Test For Ammonium:</b> To 2 ml of extract 1 ml of Nessler's reagent and excess of dil.sodium hydroxide solution are added.	No Brown colour appeared	Absence of ammonium
9.	<b>Test For Potassium:</b> A pinch (25 mg) of extract was treated of with 2 ml of dil.sodium nitrite solution and then treated with 2 ml of dil.cobalt nitrate in 30% dil.glacial acetic acid.	No Yellow precipitate was obtained	Absence of potassium
10.	<b>Test For Sodium:</b> 2 pinches (50 mg) of the extract is made into paste by using HCl and introduced into the blue flame of Bunsen burner.	No yellow colour flame evolved	Absence of sodium
11.	<b>Test For Mercury:</b> 2 ml of the extract was treated with 2 ml of dil.sodium hydroxide solution.	No Yellow precipitate obtained	Absence of Mercury
12.	<b>Test For Arsenic:</b> 2 ml of the extract was treated with 2 ml of dil.sodium hydroxide solution.	No Brownish red precipitate obtained	Absence of arsenic

S.No	EXPERIMENT	OBSERVATION	INFERENCE
<b>III. Miscellaneous</b>			
1.	<b>Test For Starch:</b> 2 ml of extract was treated with weak dil.Iodine solution	No Blue colour developed	Absence of starch
2.	<b>Test For Reducing Sugar:</b> 5 ml of Benedict's qualitative solution was taken in a test tube and allowed to boil for 2 minutes and added 8 to 10 drops of the extract and again boil it for 2 minutes. The colour changes are noted.	No Brick red colour developed	Absence of reducing sugar
3.	<b>Test For The Alkaloids:</b> a) 2 ml of the extract was treated with 2ml	Yellow colour developed	<b>Presence of Alkaloid</b>





# TLC/HPTLC FINGER PRINT ANALYSIS

## 5.4 TLC/HPTLC FINGER PRINT ANALYSIS

Thin layer chromatography (TLC) was a chromatographic technique used to separate the components of a mixture using a thin stationary phase supported by an inert backing. It may be performed on the analytical scale as a means of monitoring the progress of a reaction, or on the preparative scale to purify small amounts of a compound.

TLC/HPTLC was an analytical tool widely used because of its simplicity, relative low cost, high sensitivity, and speed of separation. TLC/HPTLC functions on the same principle as all chromatography: a compound will have different affinities for the mobile and stationary phases, and this affects the speed at which it migrates. The goal of TLC/HPTLC is to obtain well defined, well separated spots.

### TLC and HPTLC Methodology

4g of fine powdered extracted with 40ml of Ethanol soaked for 24 hours. The extract was filtered, concentrated and made up to 10 ml in a standard flask. 5 $\mu$ l, 10 $\mu$ l, 15 $\mu$ l of the solution were applied on Merck Aluminium plate pre-coated with Silica gel 60 F<sub>254</sub> of 0.2 mm thickness to a band width of 6 mm using Canag HPTLC system equipped with ATS- Iv applicator. The plate was developed in *Toluene: Ethyl acetate: Formic acid*(4:6:0.1). The plate was dried and visualized in UV 254 and UV 366 nm and photographs were taken. Before derivitization of the plate it was scanned at UV 254 nm and Fingerprint was taken before dipping in Vanillin- Sulphuric acid reagent.

### Retention Factor

After a separation was complete, individual compounds appear as spots separated vertically. Each spot has a retention factor (R<sub>f</sub>) which is equal to the distance migrated over the total distance covered by the solvent. The R<sub>f</sub> formula is,

$$\text{Rf} = \text{distance traveled by sample} / \text{distance traveled by solvent}$$

The R<sub>f</sub> value can be used to identify compounds due to their uniqueness to each compound. When comparing two different compounds under the same conditions. The compound with the larger R<sub>f</sub> value was less polar because it does not stick to the stationary phase as long as the polar compound, which would have a lower R<sub>f</sub> value.

ELEMENTAL  
ANALYSIS

## **5.5 DETERMINATION OF MICROBIAL LOAD**

Microbial analysis was carried for determination of microbial contamination as per procedures of Quality Control Methods for Medicinal plant Materials, WHO Geneva 1998 Guideline.

The test included total bacterial count, total fungal count, and identification of specified organisms such as Enterobacteriaceae, Escherichia coli, Salmonella spp, Staphylococcus aureus and Pseudomonas aeruginosa.

## **RESULTS**

The results of microbial load were represented in table 6.

## **5.6 HEAVY METAL ANALYSIS – ICP-OES**

The analysis of heavy metals and trace elements were estimated by using inductively coupled plasma optical emission spectrometry (ICP- OES). The Experimental Procedure was done at SAIF, IIT Madras, Chennai-36.

## **ICP-OESINDUCTIVELY COUPLED PLASMA OPTICAL EMISSIONS SPECTROMETRY**

### **Introduction**

The element composition of a sample is often an important part of the information needed to assess its properties. Hence there is a need for scientific instrumentation like ICP-OES which plays a pivotal role in the determination of these elements. ICP-OES is widely employed for the estimation of metals and metallolids at trace, minor and major concentration.

### **Principle**

In this technique, the high temperature plasma source atomizes the sample and excites the atoms resulting in emission of photons. The atoms of each element in the sample emit specific wavelength of light. The emission spectrum from the plasma is dispersed by an optical spectrometer , so that intension of the individual wavelength can be measured.

The number of photons emitted is directly proportional to the concentration of the element. The photons may be detected either sequentially or simultaneously.

Quantitative analysis is achieved by measuring the intensity of these specific wavelengths and after performing the calibration using known standards.

Identifying the presence of emission at the wavelength characteristic of the element of interest obtaining quantitative information i.e, how much of an element is in sample can be accomplished using plots of emission intensity versus concentration called calibration curves.

Perkin Elmer Optima 5300DV was used for standard ICP-OES analysis.

### **Sample preparation – Microwave Digestion**

- Weight 0.25 g of test sample and transfer into a liner provided with instrument.
- Slowly add 9ml of Nitric acid or sulphuric acid such that no piece of sample sticks on the slide.
- Mix thoroughly and allow reacting for few minutes.
- Place the liner in the vessel jacket.
- Close the screw cap hand- tight in clockwise direction.
- Seal the vessel and placed in the rotor fixed in microwave.
- Set temperature to 180°C for 5 minutes, hold at 180°C for least 10 minutes. Allow the vessels to cool down to a vessel interior temperature below 60°C and to a vessel surface temperature (IR) below 50°C before removing the rotor.
- The digested sample was made up to 100ml with Millipore water.
- If visible insoluble particles exist, solution could be filtered through whatmann filter paper.
- Transfer the digested solution into plastic containers and label them properly.

### **RESULT**

The analytical results of heavy metals and trace elements in *Indhuppu bhavanai* using ICP-OES were showed in table 7.

# PARTICLE SIZE ANALYSIS

## 5.7 SCANNED ELECTRON MICROSCOPY (SEM)

The particle size of the *Indhuppu bhavanai* was determined using High resolution scanning electron microscopy (HR SEM). The Experimental Procedure was done at SAIF, IIT Madras, Chennai-36.

### Experimental procedure :

A SEM is essentially a high magnification microscope, which uses a focused scanned electron beam to produce images of the sample, both top-down and, with the necessary sample preparation, cross-sections. The primary electron beam interacts with the sample in a number of key ways :-

- Primary electrons generate low energy secondary electrons, which tend to emphasize the topographic nature of the specimen.
- Primary electrons can be backscattered which produces images with a high degree of atomic number (Z) contrast.
- Ionized atoms can relax by electron shell-to-shell transitions, which lead to either X-ray emission or Auger electron ejection. The X-ray emitted are characteristic of the elements in the top few  $\mu\text{m}$  of the sample.

**Resolution** : 1.2 nm gold particle separation on a carbon substrate

**Magnification** : From a min of 12 X to greater than 1,00,000 X.

### Method :

A representative portion of each sample was sprinkled on to a double side carbon tape and mounted on aluminium stubs, in order to get a higher quality secondary electron image for SEM examination.

### Sample preparation :

Sample preparation can be minimal or elaborate for SEM analysis, depending on the nature of the samples and the data required. Minimal preparation includes acquisition of a sample that will fit into the SEM chamber and some accommodation to prevent charge build-up on electrically insulating samples.

The results were represented in figure 1.



# TOXICOLOGICAL STUDIES

## 6. TOXICOLOGICAL EVALUATION OF *INDHUPPU BHAVANAIIN*

### RODENTS

#### Introduction:

Safety was a fundamental principle in the provision of traditional medicines and herbal products for health care and a critical component of quality control. OECD guidelines provide practical and technical guidance for monitoring the safety of traditional medicines within pharmacovigilance systems. The safety monitoring of traditional medicines was compared and contrasted with that of other medicines, currently undertaken in the context of the WHO International Drug perspective.

#### Scope of work:

Monitoring Programme, while there are regulatory and cultural differences in the preparation and use of different types of medicines, they are all equally important from a pharmacovigilance

Assurance of safety, quality and efficacy of Indian System of Medicines (ISM) was the key issue that needs to be addressed while conducting toxicity studies. It was an essential step, which will strengthen the acceptance of Siddha medicines by scientific community. Information of toxicity and adverse effects of these formulations were lacking. Some of the formulations were proved to be effective in various animal studies and many more are yet to be tested.

Hence, the present study was carried out to evaluate the Preclinical animal toxicity studies of *Indhuppu bhavanaiin* rodents.

#### Plan of work:

The following studies were carried out on *Indhuppu bhavanai*.

- ❖ Acute Oral toxicity - OECD 423
- ❖ Repeated dose 28 days oral toxicity Study - OECD 407
- ❖ Repeated dose 90 days oral toxicity study - OECD 408

# ACUTE TOXICITY STUDY

## 6.1 ACUTE ORAL TOXICITY STUDY OF *INDHUPPU BHAVANAI*

### (OECD GUIDELINE – 423)

#### **Acute oral toxicity-experiment procedure:**

Acute toxicity study was carried out according to the OECD (Organization of Economic Co-operation and Development) guidelines 423. Healthy female rats, weighing 150–200g, were selected and oral administration of the single doses of *Indhuppu Bhavanai* was done aseptically by suspending in 1ml honey.

#### **Experimental animals:**

Albino rats (wistar rats) of either sex, weighing (150-200 g) were procured from animal housing facility, K.K college of Pharmacy, Gerugambakkam, Chennai. All animals were placed in a polypropylene cages in a controlled room temperature  $24^{\circ}\text{C} \pm 1^{\circ}\text{C}$  and relative humidity of 60-70 % in animal house. The animals were maintained in standard pellet diet and water ad libitum. They were acclimatized to laboratory condition for seven days before commencement of the experiment.

All the protocols and the experiments conducted in strict compliance according to ethical principles and guidelines provided by committee for the purpose of control and Supervision of Experiments on Animals ( **IAEC approval no - KKCP/2015/037**). Animal experimentation protocols are approved by Institutional Animal Ethical Committee.

#### **Administration of doses:**

*Indhuppu Bhavanai* was mixed with honey and it was administered as a single oral dose by gavage using a feeding needle. Animals were fasted prior to dosing. Following the period of fasting, the animals were weighed and then the test substance was administered. After the substance has been administered, food was withheld for further 3 to 4 hours. The principle of laboratory animal care was followed.

Observations were made and recorded systematically and continuously observed as per the guideline after substance administration.

An oral (p.o) dose of 5 mg/kg, 50 mg/kg, 300 mg/kg and 2000 mg/kg was administered step by step according to the guidelines. The general behaviors of the rats

were continuously monitored for 1 h after dosing, periodically during the first 24 h (with special attention given during the first 4 hours and then daily thereafter, for a total of 14 days. Changes in the normal psychomotor activity and external morphology and their body weights were monitored periodically before dosing and the time at which signs of toxicity or mortality were recorded.

The visual observations included skin changes, morbidity, aggressiveness, sensitivity to sound and pain, as well as respiratory movements were recorded. They were deprived of food, but not water 12 h prior to the administration of the test substance. Finally, the number of survivors were noted after 24 h and these animals were then maintained for a further 14 days and observations made daily. The toxicological effect was assessed on the basis of mortality.

Test Substance	: <i>Indhuppu Bhavanai</i>
Animal Source	: Animal house of King Institute of Preventive Medicine
Animals	: Male and Female Wistar Albino Rats
Age	: More than 8 weeks
Acclimatization	: Seven days prior to dosing.
Veterinary examination	: Prior to and at the end of the acclimatization period.
Identification of animals	: By cage number, animal number and individual marking on fur.
Diet	: Pelleted feed supplied by Godrej foods Pvt Ltd, Bangalore
Water	: Potable water in polypropylene bottles <i>ad libitum</i> .

Housing & Environment	:	The animals were housed in Polypropylene cages provided with bedding of husk.
Housing temperature	:	Between 20 & 24°C,
Relative humidity	:	Between 30% and 70%,
Dark and light cycle	:	Each of 12 hours.
Duration of the study	:	14 days

### **Number of animals and dose levels:**

Three animals were used for each step. The dose level used as the starting dose was selected from one of four fixed levels, 5, 50, 300 and 2000 mg/kg body weight. The starting dose level was most likely to produce mortality in some of the dosed animals. The available information suggests that mortality is likely at the highest starting dose level 2000mg/kg body weight, so the trial or limit test was conducted. The time interval between treatment groups is determined by the onset, duration and severity of toxic signs.

### **Observations**

Animals were observed individually after dosing at least once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first 4 hours and daily thereafter, for a total period of 14 days, except where they need to be removed from the study and humanely killed for animal welfare reasons or are found dead. It should be determined by the toxic reactions, time of onset and length of recovery period and may thus be extended when considered necessary. The times at which signs of toxicity appear and disappear are important, especially if there is a tendency for toxic signs to be delayed.

All observations were systematically recorded with individual records being maintained for each animal. Observations include changes in skin, fur, eyes, mucous membranes, respiratory, circulatory, autonomic and central nervous systems and somato motor activity and behavior pattern.

Attention was directed to observations of tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma. The principles and criteria summarized in the Humane End points Guidance Document taken into consideration. Animals found in a moribund condition and animals showing severe pain or enduring signs of severe distress was humanely killed. When animals are killed for humane reasons or found dead, the time of death should be recorded. From the maximum dose 1/5th or 1/10th of the dose was considered as therapeutic dose for further study.

**Results:**

All data were summarized in table 8.

**REPEATED DOSE 28 DAYS  
ORAL TOXICITY STUDY**



## 6.2 REPEATED DOSE 28 DAYS ORAL TOXICITY STUDY OF

### *INDHUPPU BHAVANAI* (OECD GUIDELINE - 407)

Sub-acute toxicity study was carried out according to OECD 407 and rats were divided into 3 groups of 10 animals (5 male and 5 female). *Indhuppu Bhavanai* with honey was administered to rats at the dose of 900mg /kg/day and 1800 mg/kg/day continuously for 28 days. The animals were observed daily for gross behavioural changes and other sign of sub acute toxicity. The weight of the each rat was recorded on day 0 and weekly throughout the course of the study, food and water consumption per rat was calculated. At the end of 28 days they were fasted overnight ,each animal were anaesthetized with diethyl ether and blood samples were collected from the retro-orbital plexus into two tubes: one with EDTA for immediate analysis of haematological parameters, the other without any anticoagulant and was centrifuged at 4000 rpm at 4 °C for 10 minutes to obtain the serum. Serum was stored at 20 °C until analyzed for biochemical parameters.

Test Substance	: <i>Indhuppu Bhavanai</i>
Animal Source	: Animal house of King Institute of Preventive Medicine
Animals	: Male and Female Wistar Albino Rats
Age	: More than 8 weeks
Acclimatization	: Seven days prior to dosing.
Veterinary examination	: Prior to and at the end of the acclimatization period.
Identification of animals	: By cage number, animal number and individual marking on fur.
Diet	: Pelleted feed supplied by Godrej foods Pvt Ltd, Bangalore
Water	: Portable water in polypropylene bottles ad libitum.

Housing& Environment : The animals were housed in Polypropylene cages provided with bedding of husk.

Housing temperature : Between 20 & 24°C,

Relative humidity : Between 30% and 70%,

Dark and light cycle : Each of 12 hours.

Duration of study : 28 days

#### **Justification for Dose Selection:**

The results of acute toxicity study in rats indicated that *Indhuppu Bhavanai* was non toxic and no behavioral changes were observed up to the dose level of 2000mg/kg body weight. The oral route was selected for use because oral route is considered to be a proposed therapeutic route.

#### **Preparation and administration of dose:**

*Indhuppu Bhavanai* at two doses level 900mg/kg and 1800 mg/kg respectively were prepared. The test substance was prepared every day for 7 days once for 28 days. The control animals were administered vehicle only. Administration was by oral (gavage), once daily for 28 consecutive days.

### **METHODOLOGY**

#### **Randomization, Numbering and Grouping of Animals:**

The rats randomly divided into three groups in each group consists of 10 rats (5male and 5 female) dosing up to 28 days. Animals acclimatization period of 7 days to laboratory conditions prior to the initiation of treatment. Each animal was fur marked with picric acid. The females were nulliporous and non-pregnant.

**OBSERVATIONS:** Experimental animals were kept under observation throughout the course of study for the following:

**i) Body Weight:**

Weight of each rat was recorded on day 0 and at weekly intervals throughout the course of study and at termination to calculate relative organ weights. From the data, group mean body weights and percentage of body weight gain were calculated (table -9 and table 17 )

**ii) Food Consumption:**

The quantity of food consumed by groups consisting of ten animals for different doses was recorded at weekly interval. Food consumed per animal was calculated for control and the treated dose groups. (table 11)

**iii) Clinical signs:**

All animals were observed daily for clinical signs. The time of onset, intensity and duration of these symptoms, if any, were recorded.

**iv) Mortality:**

All animals were observed twice daily for mortality during entire course of study.

**V) Laboratory investigation:**

Following laboratory investigations were carried out on day 29 in animals fasted over-night. On 29th day, the animals were fasted for approximately 18 h, then anesthetized with ether and blood samples were collected from the retro-orbital plexus into two tubes: one with EDTA for immediate analysis of haematological parameters, the other without any anticoagulant and was centrifuged at 4000 rpm at 4 °C for 10 minutes to obtain the serum. Serum was stored at 20 °C until analyzed for biochemical parameters.

**vi) Haematological Investigations:**

Blood samples of control and experimental rats were analyzed for hemoglobin, total red blood corpuscles (RBC), white blood corpuscles (WBC) count, Mean corpuscular volume (MCV) and packed cell volume (PCV). From the estimated values of RBC count (millions/mm<sup>3</sup>) and PCV (volumes percent), mean corpuscular volume (MCV) was calculated.

### **vii) Biochemical Investigations:**

Serum and Urine was used for the estimation of biochemical parameters. Samples of control and experimental rats were analyzed for protein, bilirubin, urea, uric acid, creatinine, triglyceride, cholesterol and glucose levels by using standard methods. Activities of glutamate oxaloacetate transaminase/ Aspartate aminotransferase (GOT/AST), glutamate pyruvate transaminase/ Alanine amino transferase (GPT/ALT) and alkaline phosphatase were estimated as per the colorimetric procedure.

### **viii) Necropsy:**

All the animals were sacrificed on day 29. Necropsy of all animals were carried out and the weights of the organs including liver, kidneys, adrenals, spleen, brain, heart, stomach, uterus and testes/ovaries were recorded. The relative organ weight of each animal was then calculated as follows;

Absolute organ weight (g)

Relative organ weight = \_\_\_\_\_  $\times 100$

Body weight of rats on sacrifice day (g)

### **ix) Histopathology:**

Histopathological investigation of the vital organs were done. The organ piece (3-5µm thick) of the highest dose level of 4500mg /kg were preserved and were fixed in 10% formalin for 24 h and washed in running water for 24 h. Samples were dehydrated in an auto technicon and then cleared in benzene to remove absolute alcohol. Embedding was done by passing the cleared samples through three cups containing molten paraffin at 50°C and then in a cubical block of paraffin made by the “L” moulds. It was followed by microtome and the slides were stained with Haematoxylin-eosin. The organs included heart, kidneys, liver, spleen, stomach of the animals were preserved they were subjected to histopathological examination.

**Statistical analysis:** Findings such as clinical signs of intoxication, body weight changes, food consumption, hematology and blood chemistry were subjected to One-way Anova Followed by Dunnet’s test using a computer software programme. (Graph Pad Prism 5.0). All data were summarized in tabular form (Table 9 – 17)

**REPEATED DOSE 90 DAYS  
ORAL TOXICITY STUDY**

### 6.3 REPEATED DOSE 90 DAYS ORAL TOXICITY STUDY OF

#### *INDHUPPU BHAVANAI* (OECD GUIDELINE - 408)

<b>Test Substance</b>	: <i>INDHUPPU BHAVANAI</i>
<b>Animal Source</b>	: Animal house of King Institute of Preventive Medicine
<b>Animals</b>	: Wister Albino Rats (Male -3, and Female-3)
<b>Age</b>	: 6-8 weeks
<b>Body Weight</b>	: 150-200gm.
<b>Acclimatization</b>	: Seven days prior to dosing.
<b>Veterinary examination</b>	: Prior and at the end of the acclimatization period.
<b>Identification of animals</b>	: By cage number, animal number and individual marking by using Picric acid.
<b>Diet</b>	: Pellet feed supplied by Sai meera foods Pvt Ltd, Bangalore
<b>Water</b>	: Aqua guard portable water in polypropylene bottles.
<b>Housing &amp; Environment</b>	: The animals were housed in Polypropylene cages provided with bedding of husk.
<b>Housing temperature</b>	: between 22°C $\pm$ 3°C.
<b>Relative humidity</b>	: between 30% and 70%,
<b>Air changes</b>	: 10 to 15 per hour
<b>Dark and light cycle</b>	: 12:12 hours.
<b>Duration of the study</b>	: 90 Days.

## **METHODOLOGY**

### **Randomization, Numbering and Grouping of Animals:**

24 Wistar Albino Rats (12M + 12F) were selected and divided into 4 groups. Each group consist of 6 animals (Male -3, and Female-3) **IAEC approval no NIS/IAEC – I/2016/05**. Ist group treated as a control and other three group were treated with test drug (low, mid, high) for 90 days. Animals were allowed acclimatization period of 7 days to laboratory conditions prior to the initiation of treatment. Each animal was marked with picric acid. The females were nulliparous and non-pregnant.

### **Justification for Dose Selection:**

As per OECD guideline three dose levels were selected for the study. They were low dose (X), mid dose dose (5X), high dose (10X). X is calculated by multiplying the therapeutic dose (5gm) and the body surface area of the rat (0.018). i.e X dose is 180 mg/kg, 5X dose is 900mg/kg and 10 X 1800 mg/kg of animal.

### **Preparation and Administration of Dose:**

*Indhuppu bhavanai* was suspended in Honey with distilled water to obtain concentrations of 200mg/ml. It was administered to animals at the dose levels of X, 5X, 10X. The test substance suspensions were freshly prepared every two days once for 90 days. The control animals were administered vehicle only. The drug was administered orally by using oral gavage once daily for 90 consecutive days.

## **OBSERVATIONS:**

### **Body Weight:**

Weight of each rat was recorded on day 0, at weekly intervals throughout the course of study (Table 18)

### **Clinical signs:**

All animals were observed daily for clinical signs. The time of onset, intensity and duration of these symptoms, if any, were recorded.

**Mortality:**

All animals were observed twice daily for mortality during entire course of study.

**Laboratory Investigations:**

Following laboratory investigations were carried out on day 91 in animals fasted over-night. Blood samples were collected from orbital sinus using sodium heparin (200IU/ml) for Bio chemistry and potassium EDTA (1.5 mg/ml) for Haematology as anticoagulant. Blood samples were centrifuged at 3000 r.p.m. for 10 minutes.

**Haematological Investigations:**

Haematological parameters were determined using Haematology analyzer.

**Biochemical Investigations:**

Biochemical parameters were determined using auto-analyzer.

**Histopathology:**

Control and highest dose group animals will be initially subjected to histopathological investigations. If any abnormality found in the highest dose group than the low, then the mid dose group will also be examined. Organs will be collected from all animals and preserved in 10% buffered neutral formalin for 24 h and washed in running water for 24 h. The organ sliced 5 or 6µm sections and were dehydrated in an auto technicon and then cleared in benzene to remove absolute alcohol. Embedding was done by passing the cleared samples through three cups containing molten paraffin at 50°C and then in a cubical block of paraffin made by the “L” moulds. It was followed by microtome and the slides were stained with Haematoxylin-eosin.

**Statistical analysis:**

Findings such as clinical signs of intoxication, body weight changes, haematology and blood chemistry were subjected to One-way ANOVA followed by dunnet't test using a computer software programme -INSTAT-V3 version. All data were summarized in tabular form (Table 18–23 ).



# PHARMACOLOGICAL STUDY

# ANTI-ULCER ACTIVITY

## 7.1 ANTIULCER ACTIVITY OF *INDHUPPU BHAVANAION*

### WISTAR ALBINO RATS

#### Aim:

To evaluate the antiulcer activity of *Indhuppu bhavanai* in Wistar albino rats by *Aspirin induced ulcer model*.

#### Selection of Experimental animals:

Healthy Wistar albino rats of either sex weighing (150-200 gms ) were used for this study. The animals were obtained from animal house, K.K college of pharmacy, Gerugambakkam, Chennai. Animals were housed at a temperature of  $24 \pm 2^{\circ} \text{C}$  and relative humidity of 30-70% at 12 :12 light, day cycle was followed. All the animals were allowed to free access to water and fed with standard commercial pellet. All the experimental procedures and protocols used in this study were reviewed by (IAEC) Institutional Animal Ethics Committee (**IAEC approval No KKCP/2015/037**) of K.K college of Pharmacy and were in accordance with the guidelines of the IAEC.

#### Aspirin Induced Gastric Ulcers

#### Principle:

Aspirin was a NSAID which inhibit the synthesis of prostaglandins. Prostaglandins protect the gastric mucosa by producing leukotrienes and bicarbonate ions. Aspirin also inhibit the gastric peroxidase and may increase mucosal hydrogen peroxide and hydroxyl ions level to cause oxidative mucosal damage.

#### Procedure:

Albino rats of either sex weighing between 150-250 gms were divided into five groups and each consisting of six rats.

- Group I : Control (2ml/kg p.o.)
- Group II : Negative control received only Aspirin (500mg/kg)
- Group III : Standard drug Ranitidine(20mg/kg)+ Aspirin (500 mg/kg)
- Group IV : Received *Indhuppu bhavanai* 200mg/kg + Aspirin (500 mg/kg)
- Group V : Received *Indhuppu bhavanai* 400mg/kg + Aspirin (500 mg/kg)

The animals were fasted for 24 hours. The test drug in varying concentration based on the design of the experiment is administered orally 30 minute prior to aspirin at dose of 500 mg/kg. 4hours later the rats were scarified by using anaesthetic ether and their stomachs were dissected and they were opened along the greater curvature for the determination of gastric lesions. Ulcer index was calculated by the number of ulcers per animal and severity scored by observing the ulcers microscopically with the help of 10X lens.

### **Evaluation of parameters:**

#### **Effect of Free Acidity and Total Acidity :**

The free acidity and total acidity was determined based on the titre values. One ml of gastric juice was pipetted into 100 ml of conical flask and titrates with 0.01N NaOH using toppers reagent as an indicator (It is Dimethyl –amino-azo-benzene with phenolphthalein and used for the detection and estimation of hydrochloric acid and total acidity in gastric fluids) titrate to end point, when the solution turns to orange colour, note the volume of NaOH which corresponds to free acidity. Titrate further till the solution regains its pink colour. Note the volume of NaOH which corresponds to the free acidity. Acidity (mEq/L/100g) can be expressed as

$$\text{Acidity} = \frac{\text{Volume of NaOH} \times \text{Normality of NaOH}}{0.1} \times 100$$

#### **Ulcer index :**

The ulcer index was calculated by taking the mean ulcer score of each groups. Then the mean ulcer score graph was plotted with groups on x-axis and ulcer index on y-axis. The histograms of different groups were then interpolated by comparing the ulcer index of groups

#### **Collection of Gastric Juice**

The stomach was excised carefully opened along the greater curvature and the gastric contents were removed. The gastric contents were collected in plain tubes and centrifuged at 3000 rpm for 5 min, the volume of the supernatant was expressed as ml /100

gm body weight. The mucosa was flushed with saline and observed for gastric lesions using a dissecting microscope, ulcer score was determined.

### **Ulcer Scoring**

After sacrificing the rat, stomach was removed and opened along the greater curvature, and washed it slowly under running tap water. Put it on the glass slide and observe under 10X magnification for ulcer. Score the ulcers as below.

0	=	normal coloured stomach
0.5	=	red colouration
1	=	spot ulcers
1.5	=	haemorrhagic streaks
2	=	Ulcers $\geq 3$ but $\leq 5$
3	=	Ulcers $>5$

Mean ulcer score for each animal is expressed as Ulcer Index.

The results of anti-ulcer activity by Aspirin induced ulcer model showed in table - 26

# ANTI-INFLAMMATORY ACTIVITY

## 7.2 ANTI- INFLAMMMATORY ACTIVITY OF *INDHUPPU* *BHAVANAI* ON WISTAR ALBINO RATS

### AIM:

To evaluate the anti - inflammatory activity of *Indhuppu bhavanai* in Wistar albino rats by Cotton pellet granuloma method.

### Selection of Experimental animals:

The experimental protocol was submitted and approved by institutional Ethical Committee, (IAEC approval No KKCP/2015/037). Wistar albino rats (150- 180 gm) of approximate same age were employed in this investigation. The animals were obtained from animal house, K.K college of pharmacy, gerugambakkam, Chennai. Animals were housed at a temperature of  $24\pm 2^{\circ}$  C and relative humidity of 30-70% at 12 :12 light, day cycle was followed. All the animals were allowed to free access to water and fed with standard commercial pellet.

### Experimental Design for Cotton pellet granuloma model

The animals were divided into four groups each group consists of 6 animals.

- Group-I : Control - Vehicle control received distilled water (dose: 10 ml/kg).
- Group-II : Standard drug - Animals treated with Dexamethasone (dose: 0.5 mg/kg).
- Group-III : Animals treated with *Indhuppu Bhavanai* ( 200 mg/kg).
- Group-IV : Animals treated with *Indhuppu Bhavanai* ( 400 mg/kg)

### Experimental procedure

Inflammation was induced by cotton pellet granuloma model. This method was carried out by using sterilized cotton pellet implantation method in rats. Under light ether anesthesia by using blunted forceps, subcutaneous tunnel was made and sterilized cotton pellets ( $10 \pm 1$  mg) were implanted in the axilla and groin region of the rat. After recovering from anaesthesia, animals were treated orally with vehicle control (Distilled water 10 ml / kg), Dexamethasone 0.5 mg/kg, low dose (200mg/kg) and high dose (400mg/kg) of *Indhuppu Bhavanai* for consecutive 7 days, once per day. They were

sacrificed on day 8th by cervical dislocation and the pellets were removed and immediately the wet weight was taken, freed from extraneous tissue and dried at 60°C for 24 hrs. The percentage inhibition of wet weight and dry weight of the granuloma were calculated and compared.

$$\text{Percentage inhibition (\%)} = \frac{\text{Control} - \text{Treated}}{\text{Control}} \times 100$$

The results of anti-inflammatory activity by Cotton pellet granuloma method showed in table - 27.



# ANALGESIC ACTIVITY

### **7.3 ANALGESIC ACTIVITY OF *INDHUPPU BHAVANAI* ON SWISS ALBINO MICE**

#### **AIM:**

To evaluate the Analgesic activity of *Indhuppu bhavanai* in Wistar albino rats by *Eddy's Hot plate method*.

#### **Selection of Experimental animals:**

The experimental protocol was submitted and approved by institutional Ethical Committee, (IAEC approval No KKCP/2015/037). Swiss albino mice (20-25 gm) of approximate same age were employed in this investigation. The animals were obtained from animal house, K.K College of Pharmacy, Gerugambakkam, Chennai. Animals were housed at a temperature of  $24\pm 2^{\circ}\text{C}$  and relative humidity of 30-70% at 12 :12 light, day cycle was followed. All the animals were allowed to free access to water and fed with standard commercial pellet.

#### **Evaluation of Analgesic activity**

Pain is the part of a defensive reaction against dysfunction of an organ or imbalance in its functions against potentially dangerous stimulus. The ascending pathway of pain includes the contra lateral spinothalamic tract, lateral pons, mid brain to thalamus and ultimately through the somatosensory cortex of the brain that determines the locations, intensity and depth of pain

#### **Eddy's Hot plate method:**

##### **Principle:**

Painful reactions can be produced in experimental animals by applying noxious stimuli such as thermal – using radiant heat as a source of pain, chemical – using irritants such as acetic acid and bradykinin and physical pressure – using tail compression.

The hot plate test was a test of the pain response in animals. It was used in basic pain research and in testing the effectiveness of analgesics by observing the reaction to pain caused by heat.

They used a behavioral model of nociception where behaviors such as jumping and hind paw-licking are elicited following a noxious thermal stimulus. Licking was a rapid response to painful thermal stimuli that was a direct indicator of nociceptive threshold. Jumping represents a more elaborated response, with a latency and encompasses an emotional component of escaping.

### **Animals**

Mice 20-25 g were grouped in four groups, six animals in each group.

### **Grouping:**

**Group I** : Control - distilled water (10ml/kg, p.o.),

**Group II** : Standard drug - Pentazocine (5mg/kg, p.o.)

**Group III** : *Indhuppu Bhavannai* (200mg/kg)

**Group IV** : *Indhuppu Bhavannai* (400mg/kg)

### **Equipment:**

Eddy's Hot plate

### **Procedure:**

Animals were weighed and placed on the hot plate. Temperature of the hot plate was maintained at  $55 \pm 1^\circ \text{C}$ . Jumping response was seen. The time period (latency period), from when the animals were placed and until the responses occurred, were recorded using a stopwatch. To avoid tissue damage of the animals 10 seconds was kept as a cut off time. The time obtained was considered the basal / normal reaction time in all the untreated groups of animals. Increase in the basal reaction time was the index of analgesia.

All the animals were screened initially at least three times in this way and the animals showing a large range of variation in the basal reaction time were excluded from the study. A final reading of the basal reaction time was recorded for the included animals. After selecting the animals, the drugs were administered to all the groups at the stipulated doses. The reaction times of the animals were then noted at 0, 30, 60, 90, 120 and 150 mins interval after drug administration.

### **Statistical analysis**

Results were expressed as mean  $\pm$  SEM and analyzed using Graph Pad Prism software. One way analysis of variance (ANOVA) test was applied.

P value less than 0.05 ( $P < 0.05$ ) was considered as statistically significant.

The results of analgesic activity by Eddy's Hot plate method was represented in table 28.

# RESULTS

## 8 RESULTS

### 5.1 ORGANOLEPTIC CHARACTER\*

**Table:1. Organoleptic characters of *Indhuppu bhavanai***

Colour	Dark Brown
Odour	Characteristic odour
Taste	Characteristic taste
Texture	Fine powder

### 5.2 PHYSICOCHEMICAL ANALYSIS\*

**Table 2 Physicochemical properties of *Indhuppu bhavanai***

S.NO	Parameters	Results
1.	pH at 10% of aqueous solution	3.91
2.	Total Ash	62.12%
3.	Water soluble extractive	45.52%
4.	Acid insoluble ash	0.1%
5.	Loss on drying @ 105° C(w/w)	6.04%
6	Alcohol soluble extractive	8.92

---

\*The experimental procedure was analyzed at Captain Srinivasamurti Research Institute for Ayurveda and Siddha Drug Development, Arumbakkam, Ch-106.

### 5.3 CHEMICAL ANALYSIS \*

**Table 3: Results of acid radicals studies of *Indhuppu bhavanai***

S.NO	Parameter	Observation	Result
1	Test for Sulphate	Cloudy appearance present	<b>Positive</b>
2	Test for Chloride	Cloudy appearance present	<b>Positive</b>
3	Test For Phosphate		Negative
4	Test For Carbonate		Negative
5	Test For Nitrate	-	Negative
6	Test for Sulphide	-	Negative
7	Test For Fluoride & oxalate	-	Negative
8	Test For Nitrite	-	Negative
9	Test For Borax	-	Negative

#### **Interpretation**

The acidic radicals test shows the presence of **Chloride** and **sulphate**.

---

\*The chemical analysis was carried out in Bio chemistry Lab, NIS, Ch – 47

**Table: 4 Results of basic radicals studies of *Indhuppu bhavanai*\***

S.NO	Parameter	Observation	Result
1	Test for Lead	-	Negative
2	Test for Copper	-	Negative
3	Test For Aluminium	-	Negative
4	Test For Iron	Blood red colour	<b>Positive</b>
5	Test For Zinc	-	Negative
6	Test for Calcium	Cloudy appearance and white precipitate is obtained	<b>Positive</b>
7	Test For Magnesium	-	Negative
8	Test For Ammonium	-	Negative
9	Test For Potassium	-	Negative
10	Test For Sodium	-	Negative
11	Test For Mercury	-	Negative
12	Test For Arsenic	-	Negative

### **Interpretation**

The basic radical test shows the presence of **Iron, Calcium** and absence of heavy metals such as lead, arsenic and mercury.

---

\*The chemical analysis was carried out in Bio chemistry Lab, National Institute Of Siddha, Ch- 47



#### 5.4 TLC and HPTLC analysis of *Indhuppu bhavanai* \*

The procedure recommended for the analysis of TLC and HPTLC analysis as per Wagner H and Bladt S, 1996

**Table 5:**

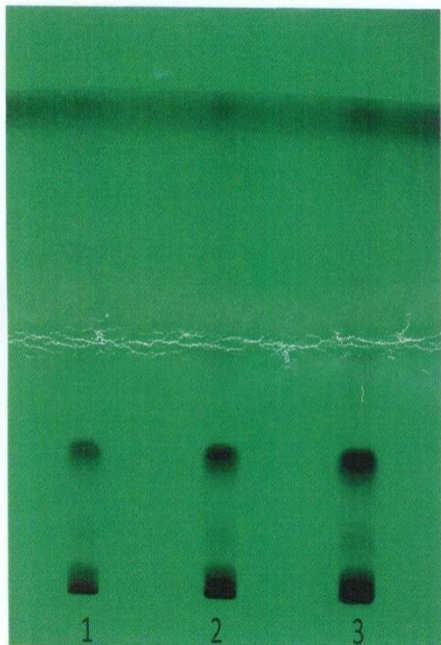
S.No	254nm		366nm		Dipped in Vanillin-Sulphuric Acid	
	Colour	Rf	Colour	Rf	Colour	Rf
1	Green	0.08	Red	0.54	Grey	0.08
2	Green	0.34	Blue	0.86	Grey	0.23
3	Green	0.97	Blue	0.91	Red orange	0.27
4			Red	0.98	Grey	0.47
5					Grey	0.81
6					Grey	0.86
7					Brown	0.97

---

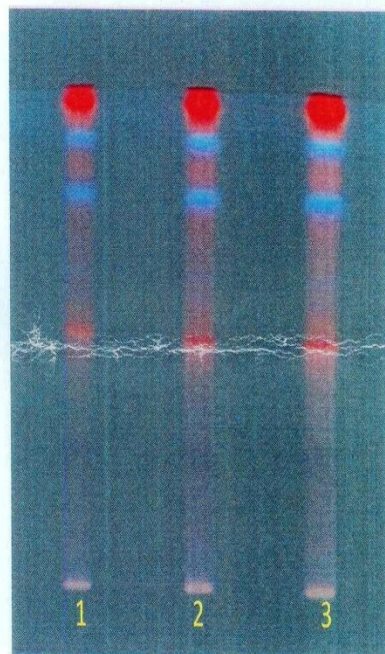
\*The experimental procedure was analyzed at Captain Srinivasamurti Research Institute for Ayurveda and Siddha Drug Development, Arumbakkam, Ch-106.

**TLC Photo documentation of DTL 1510354 Sample (Ethanol extract)**

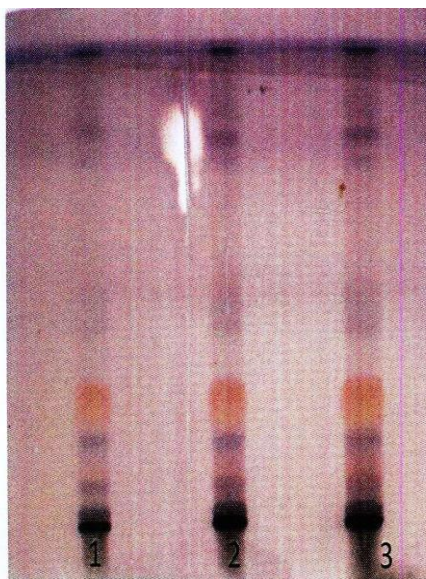
**254nm**



**366nm**



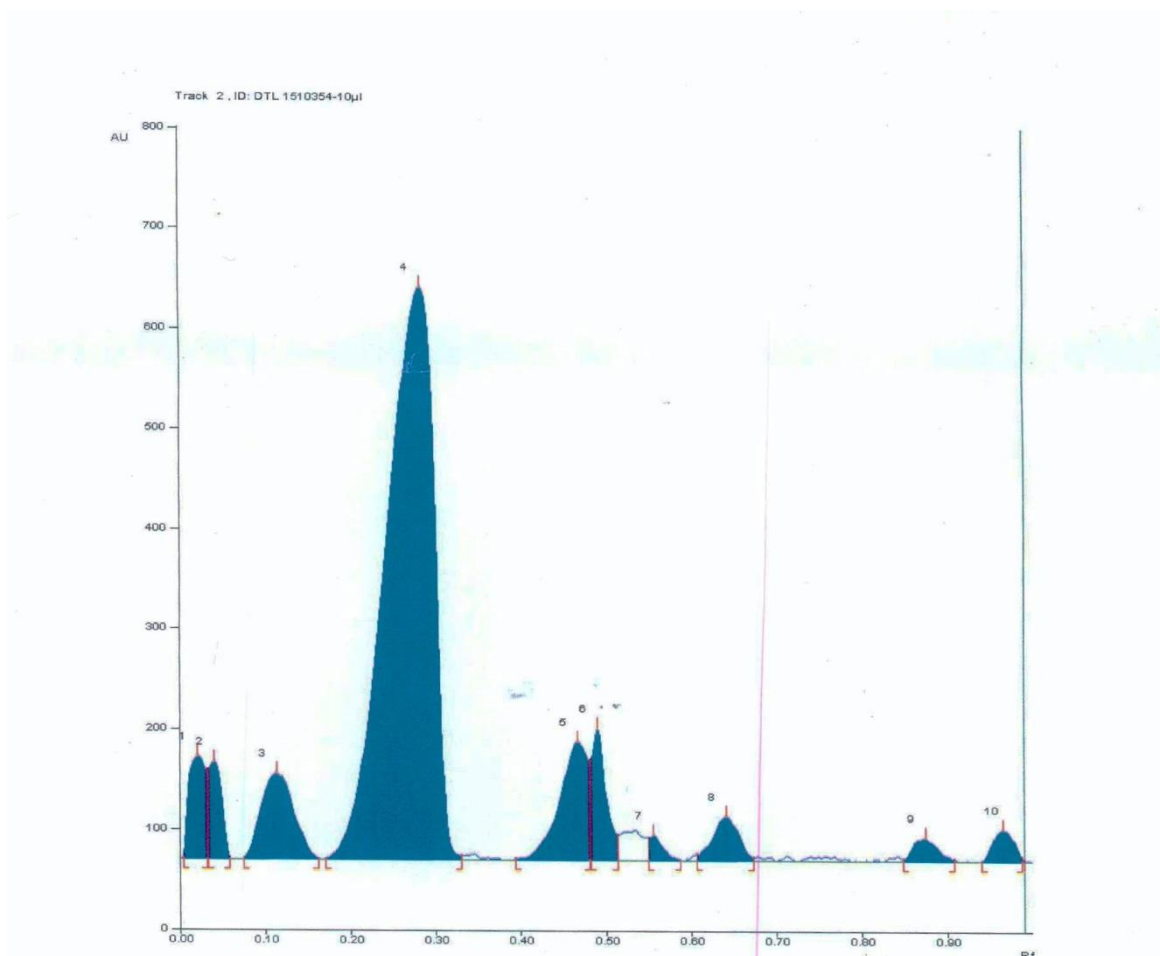
**After dipping vanillin sulphuric acid**



**Solvent system : Toluene: Ethyl acetate: formic acid (4:6:0.1)**

**Track 1-5 µl; Track 2-10µl; Track 3-15µl**

## HPTLC finger print profile of DTL 1510354 sample (Ethanol extract)-5µl -254nm

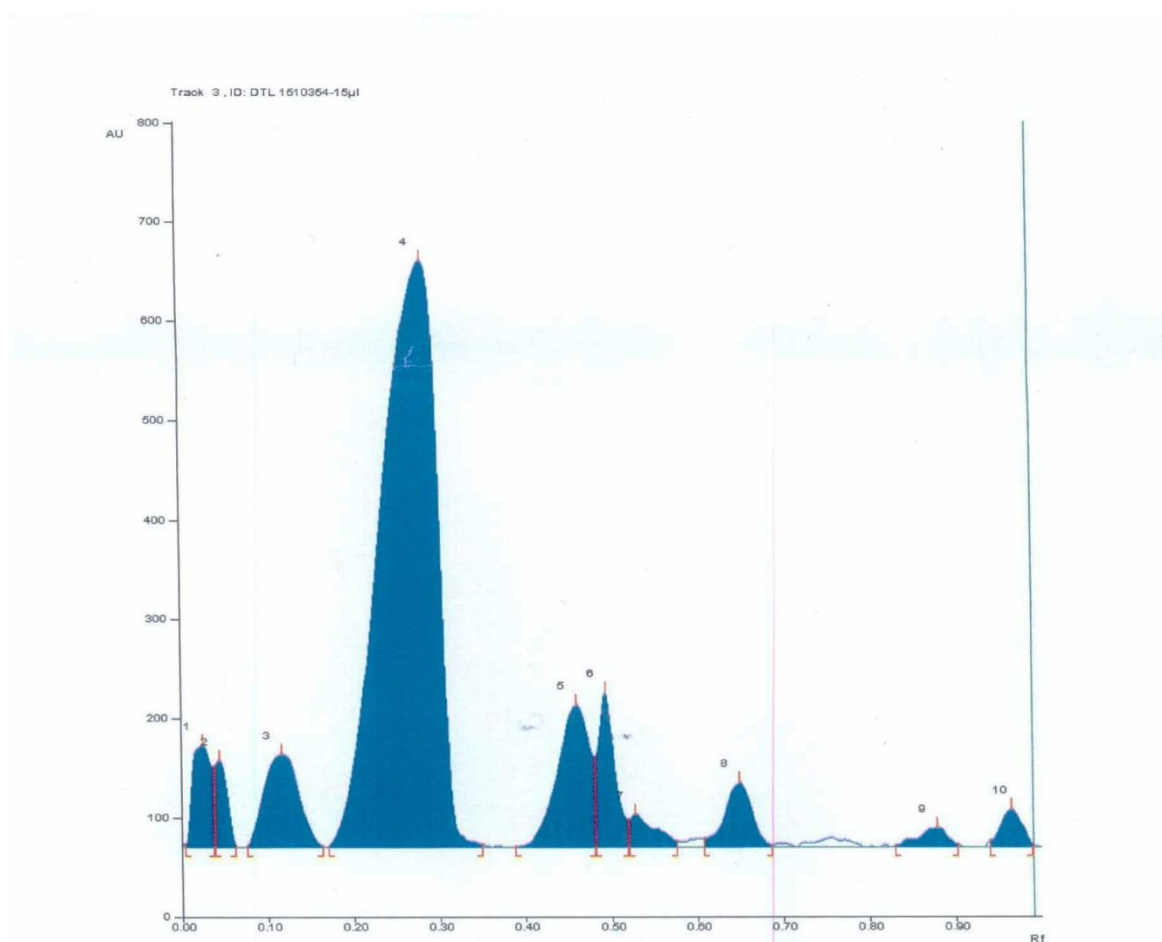


Track 2, ID: DTL 1510354-10µl

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	0.00 Rf	2.9 AU	0.02 Rf	102.4 AU	8.34 %	0.03 Rf	38.4 AU	1587.6 AU	4.07 %	unknown *
2	0.03 Rf	88.8 AU	0.04 Rf	97.1 AU	7.91 %	0.06 Rf	0.8 AU	1240.0 AU	3.18 %	unknown *
3	0.07 Rf	0.6 AU	0.11 Rf	85.2 AU	6.94 %	0.16 Rf	0.3 AU	2632.0 AU	6.74 %	unknown *
4	0.17 Rf	0.3 AU	0.28 Rf	571.1 AU	46.52 %	0.33 Rf	4.2 AU	26074.7 AU	66.78 %	unknown *
5	0.39 Rf	0.6 AU	0.47 Rf	118.2 AU	9.63 %	0.48 Rf	39.0 AU	3144.7 AU	8.05 %	unknown *
6	0.48 Rf	99.9 AU	0.49 Rf	131.3 AU	10.69 %	0.51 Rf	25.3 AU	1797.5 AU	4.60 %	unknown *
7	0.55 Rf	22.4 AU	0.56 Rf	25.1 AU	2.04 %	0.59 Rf	0.2 AU	345.5 AU	0.88 %	unknown *
8	0.61 Rf	6.0 AU	0.64 Rf	43.5 AU	3.54 %	0.67 Rf	2.8 AU	1072.7 AU	2.75 %	unknown *
9	0.85 Rf	1.8 AU	0.88 Rf	22.7 AU	1.85 %	0.91 Rf	1.8 AU	528.3 AU	1.35 %	unknown *
10	0.94 Rf	0.2 AU	0.97 Rf	31.1 AU	2.54 %	0.99 Rf	4.6 AU	622.3 AU	1.59 %	unknown *



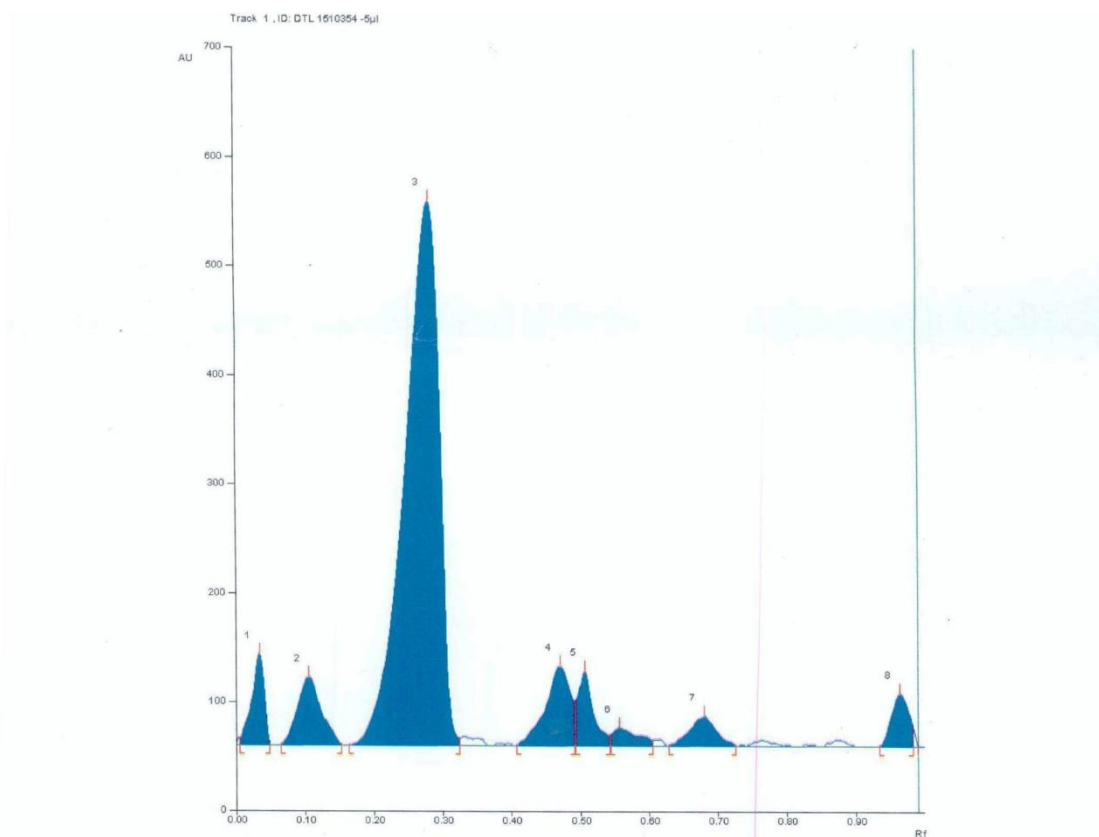
## HPTLC finger print profile of DTL 1510354 sample (Ethanol extract)-10µl-254nm



Track 3, ID: DTL 1510354-15µl

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	0.00 Rf	2.3 AU	0.02 Rf	102.1 AU	7.71 %	0.04 Rf	30.2 AU	1771.9 AU	3.74 %	unknown *
2	0.04 Rf	81.0 AU	0.04 Rf	87.1 AU	6.58 %	0.06 Rf	1.0 AU	1002.9 AU	2.12 %	unknown *
3	0.08 Rf	0.6 AU	0.12 Rf	92.7 AU	7.00 %	0.16 Rf	0.3 AU	3032.7 AU	6.40 %	unknown *
4	0.17 Rf	0.4 AU	0.28 Rf	590.6 AU	44.59 %	0.35 Rf	2.3 AU	31147.0 AU	65.75 %	unknown *
5	0.39 Rf	0.1 AU	0.46 Rf	143.0 AU	10.79 %	0.48 Rf	39.6 AU	4345.1 AU	9.17 %	unknown *
6	0.48 Rf	89.9 AU	0.50 Rf	155.4 AU	11.73 %	0.52 Rf	27.4 AU	2387.7 AU	5.04 %	unknown *
7	0.52 Rf	27.6 AU	0.53 Rf	32.8 AU	2.48 %	0.58 Rf	5.6 AU	789.9 AU	1.67 %	unknown *
8	0.61 Rf	8.0 AU	0.65 Rf	64.5 AU	4.87 %	0.69 Rf	1.3 AU	1640.0 AU	3.46 %	unknown *
9	0.83 Rf	1.2 AU	0.88 Rf	19.0 AU	1.43 %	0.90 Rf	2.1 AU	508.9 AU	1.07 %	unknown *
10	0.94 Rf	6.1 AU	0.97 Rf	37.3 AU	2.82 %	0.99 Rf	2.0 AU	749.0 AU	1.58 %	unknown *

## HPTLC finger print profile of DTL 1510354 sample (Ethanol extract)- 15µl -254nm



Track 3, ID: DTL 1510354-15µl

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	0.00 Rf	2.3 AU	0.02 Rf	102.1 AU	7.71 %	0.04 Rf	30.2 AU	1771.9 AU	3.74 %	unknown *
2	0.04 Rf	81.0 AU	0.04 Rf	87.1 AU	6.58 %	0.06 Rf	1.0 AU	1002.9 AU	2.12 %	unknown *
3	0.08 Rf	0.6 AU	0.12 Rf	92.7 AU	7.00 %	0.16 Rf	0.3 AU	3032.7 AU	6.40 %	unknown *
4	0.17 Rf	0.4 AU	0.28 Rf	590.6 AU	44.59 %	0.35 Rf	2.3 AU	31147.0 AU	65.75 %	unknown *
5	0.39 Rf	0.1 AU	0.46 Rf	143.0 AU	10.79 %	0.48 Rf	39.6 AU	4345.1 AU	9.17 %	unknown *
6	0.48 Rf	89.9 AU	0.50 Rf	155.4 AU	11.73 %	0.52 Rf	27.4 AU	2387.7 AU	5.04 %	unknown *
7	0.52 Rf	27.6 AU	0.53 Rf	32.8 AU	2.48 %	0.58 Rf	5.6 AU	789.9 AU	1.67 %	unknown *
8	0.61 Rf	8.0 AU	0.65 Rf	64.5 AU	4.87 %	0.69 Rf	1.3 AU	1640.0 AU	3.46 %	unknown *
9	0.83 Rf	1.2 AU	0.88 Rf	19.0 AU	1.43 %	0.90 Rf	2.1 AU	508.9 AU	1.07 %	unknown *
10	0.94 Rf	6.1 AU	0.97 Rf	37.3 AU	2.82 %	0.99 Rf	2.0 AU	749.0 AU	1.58 %	unknown *

**Table : 6 Screening for Micro – organisms in *Indhuppu bhavanai* \***

S.No	Parameters	Results	Permissible Limit for Internal use
1	Total Bacterial Count (TBC)	<10 <sup>3</sup> cfu/g	10 <sup>5</sup> CFU/g
2	Total Fungal Count (TBC)	<10 <sup>3</sup> cfu/g	10 <sup>3</sup> CFU/g
3	Enterobacteriaceae	Absent	10 <sup>3</sup> CFU/g
4	<i>Escherichia coli</i>	Absent	10CFU/g
5	<i>Salmonella spp</i>	Absent	Absent
6	<i>Staphylococcus aureus</i>	Absent	Absent

## RESULTS

The microbial load analysis confirms *Indhuppu bhavani* was free from micro-organisms and fungal infections.

---

\*The experimental procedure was analyzed at Captain Srinivasamurti Reseach Institute for Ayurveda and Siddha Drug Development, Arumbakkam, Ch-106.

## 5.6 ELEMENTAL ANALYSIS

**Table 7** ICP-OES study results of *Indhuppu bhavanai*

S.No	Elements	Wavelength in nm	mg/L
1	Arsenic	As188.979	BDL
2	Calcium	Ca 315.807	12.460
3	Cadmium	Cd 228.802	BDL
4	Copper	Cu 327.393	BDL
5	Mercury	Hg 253.652	BDL
6	Potassium	K 766.491	13.801
7	Sodium	Na 589.592	124.310
8	Nickel	Ni 231.604	BDL
9	Lead	220.353	BDL
10	Phosphorus	P 213.617	06.741

**BDL - Below Detection Limit, ppm – Parts per million**

**Table 7** shows the quantitative analysis of the elements present in *Indhuppu bhavanai*. The heavy metals were found to be within normal limits. The presence of other elements shows the therapeutic value of *Indhuppu bhavanai*.

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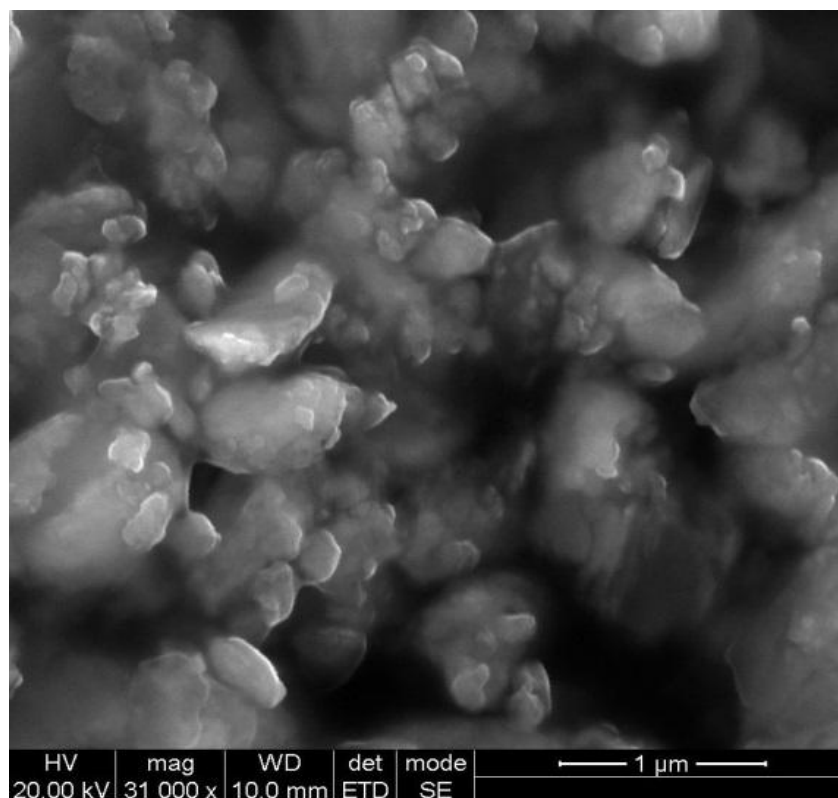
\* The Experimental Procedure was done at SAIF, IIT Madras, Chennai-36.

## 5.7 PARTICLE SIZE ANALYSIS \*

### SCANNED ELECTRON MICROSCOPY

#### Determination Of Particle size of *Indhuppu bhavanai*

Figure 1



The picture shows that the particles are stabilize, have irregular morphology and distributed in near nano range. *Indhuppu bhavanai* has particle size of 0.06 to 0.15 $\mu$ .

---

\*The Experimental Procedure was done at SAIF, IIT Madras, Chennai-36.



## 6 TOXICITY STUDY ON *INDHUPPU BHAVANAI*

### 6.1 Acute oral toxicity study of *Indhuppu bhavanai* \*

**Table 8: Dose finding experiment and its behavioural Signs of Toxicity in wistar albino rats**

No	Dose mg/kg	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	5	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	50	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	300	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	2000	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-

1.Alertness 2.Aggressive 3.Pile erection 4.Grooming 5.Gripping 6.Touch Response  
7.Decreased Motor activity 8.Tremors 9.Convulsions 10.Muscle Spasm 11.catatonia  
12.Muscle relaxant 13.Hypnosis 14.Analgesia 15.Lacrimation 16.Exophthalmos  
17.Diarrhoea 18. Writhing 19.Respiration 20.Mortality

**+ Presence of Activity**

**-Absence of Activity**

### RESULT

All the data were summarized in the form of table (8) revealed no abnormal signs and behavioral changes in rats upto the dose level of 2000 mg/kg body weight administered orally

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\* Acute oral toxicity was done at K.K college of Pharmacy, Gerugambakkam, Chennai

**Table 9 : Body wt gram of wistar albino rats exposed to Indhuppu Bhavanai for 28 days**

Dose (mg/kg/day)	Days				
	0	7	14	21	28
<b>Control</b>	157±4.37	157.42±1.17	156.92±1.21	157.36±1.41	158.01±1.03
<b>MD dose</b>	154.13±0.82	154.72±0.55	155.24±0.98	156.1±1.17	159.12±0.52
<b>HD dose</b>	155.8±1.03	156.12±0.75	156.69±0.89	157.22±0.75	158.11±1.52

Values are mean of 10 animals ± S.E.M (Dunnet's test) \*p<0.05 ;\*\*p<0.01.N=10

**Table 10 :Water (ml/day) intake of albino rats exposed to Indhuppu Bhavanai for 28 days**

Dose(mg/kg/day)	Days				
	1	7	14	21	28
<b>Control</b>	32.0±0.92	33.36±0.23	33.23±0.23	33.18±0.16	33.64±0.38
<b>MD dose</b>	35.83±0.49	34.5±1.52	34.33±1.51	35.5±1.05	35.83±1.72
<b>HD dose</b>	34.72±1.51	35.19±1.79	36.17±1.51	36.42±1.33	36.59±1.52

Values are mean of 10 animals ± S.E.M (Dunnet's test) \*p<0.05 ;\*\*p<0.01.N=10

**Table 11: Food (g/day) intake of albino rats exposed to Indhuppu Bhavanai for 28 days**

Dose (mg/kg/day)	Days (gms/rats)				
	1	7	14	21	28
<b>Control</b>	33.5±0.84	34.72±1.17	35±0.75	35.32±1.97	35.86±1.37
<b>MD dose</b>	35.82±0.82	38.16±0.75	38.02±0.98	39.83±1.60	39.33±1.21
<b>HD dose</b>	34.75±2.02	37.13±1.33	37.93±1.10	39.33±1.51	39.83±1.17

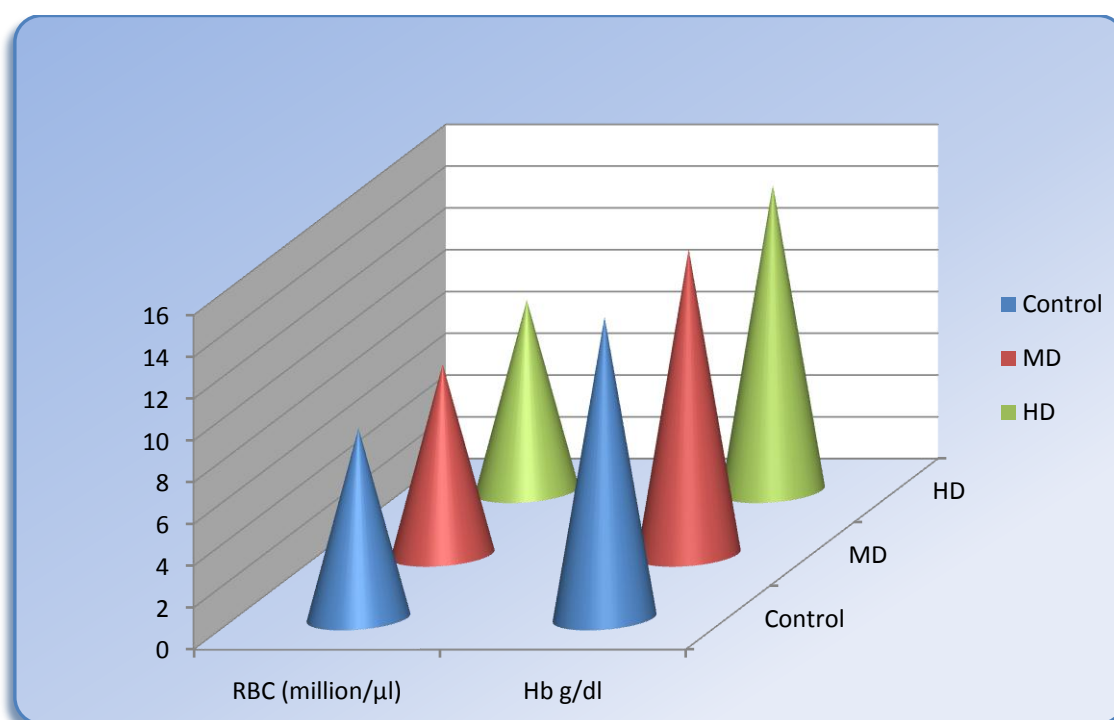
Values are mean of 10 animals ± S.E.M (Dunnet's test)\* p<0.05 ;\*\*p<0.01.N=10

**Table 12: Hematological parameters after 28days treatment with *Indhuppu Bhavanai* in rats**

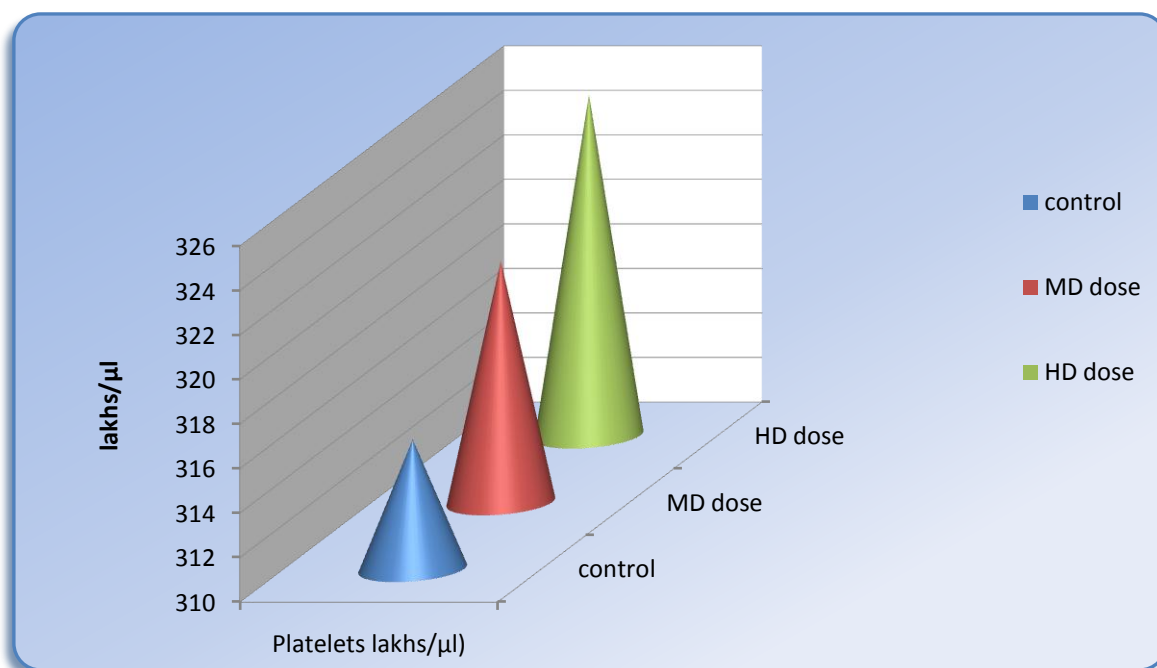
Parameters	Control	MD dose	HD dose
Total RBC( $10^6\text{mm}^{-3}$ )	9.08 $\pm$ 0.03	9.09 $\pm$ 0.02	9.11 $\pm$ 0.02
HB (g/dl)	14.40 $\pm$ 0.18	14.60 $\pm$ 0.32	14.63 $\pm$ 0.29
Leukocyte( $10^3\text{mm}^{-3}$ )	12.33 $\pm$ 0.22	12.13 $\pm$ 0.12	12.16 $\pm$ 0.15
Platelets lakhs/ $\mu\text{l}$	315.81 $\pm$ 4.43	320.83 $\pm$ 0.98	325.30 $\pm$ 3.18
MCV%ft	51.01 $\pm$ 0.76	52.62 $\pm$ 1.22	52.21 $\pm$ 1.18
PCV%	39.13 $\pm$ 0.12	39.24 $\pm$ 0.37	39.45 $\pm$ 1.22

Values are mean of a 10 animals  $\pm$  S.E.M (Dunnet's test)\*  $p < 0.05$  ; \*\* $p < 0.01$ . N=10

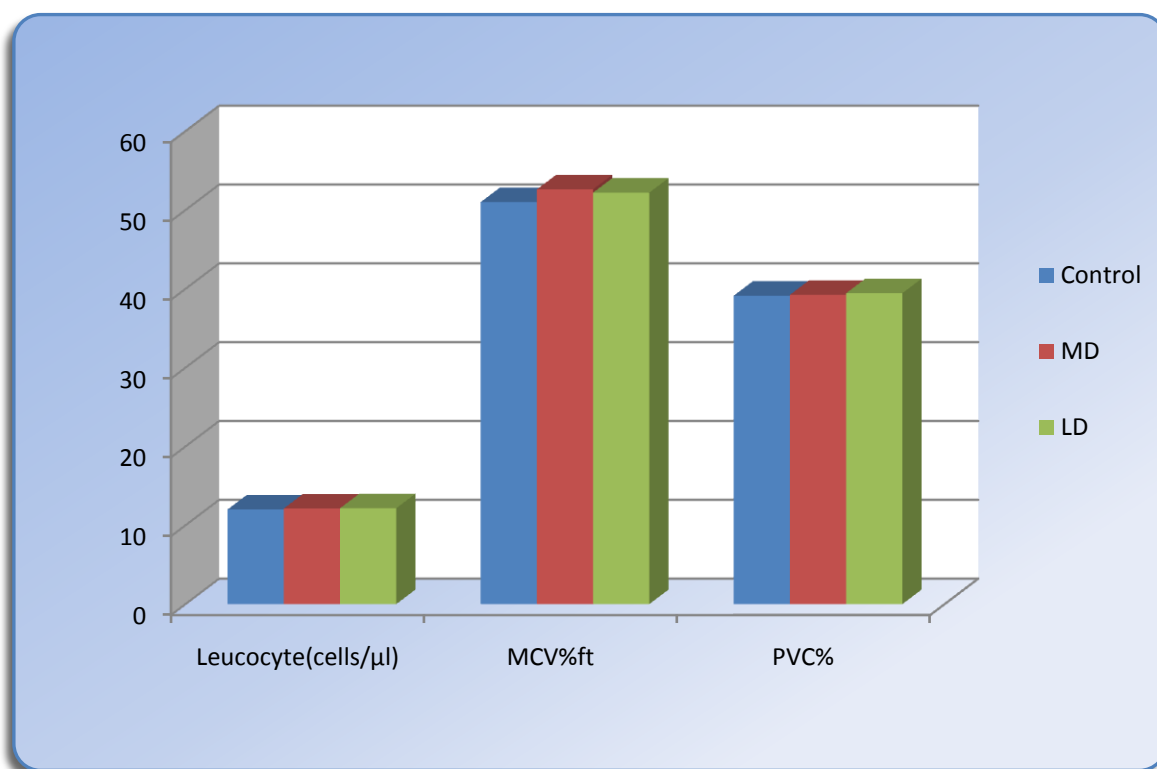
**Chart 1: The mean value of HB, Total RBC of control and treated groups of wistar albino rats exposed to *Indhuppu bhavanai* for 28 days**



**Chart 2 : The mean value of Platelet of control and treated groups of wistar albino rats exposed to *Indhuppu bhavanai* for 28 days**



**Chart 3: The mean value of Leucocyte, MCV and PCV of control and treated groups of wistar albino rats exposed to *Indhuppu bhavanai* for 28 days**

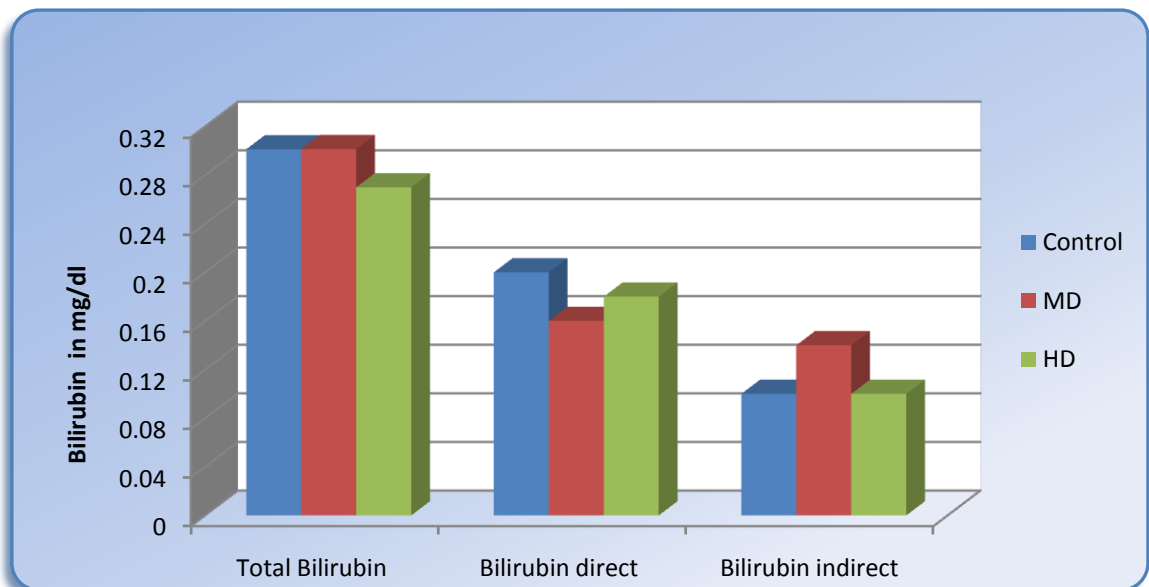


**Table 13 : Effect of treatment with *Indhuppu Bhavanai* on biochemical parameters**

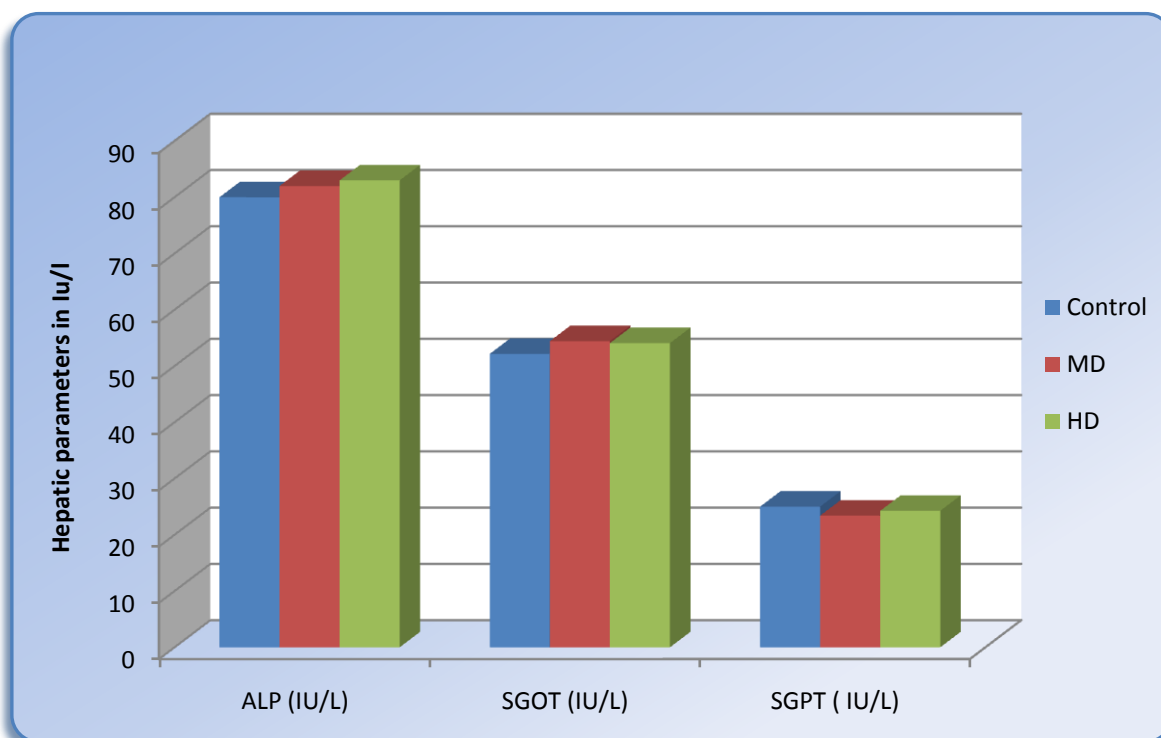
Dose(mg/kg)	Control	MD dose	HD dose
<b>Total Bilirubin(mg/dl)</b>	0.3013±0.01	0.3015±0.01	0.27±0.01
<b>Bilirubin direct(mg/dl)</b>	0.2±0.02	0.16±0.02	0.18±0.02
<b>Bilirubin indirect(mg/dl)</b>	0.1±0.02	0.14±0.01	0.1±0.01
<b>ALP(IU/L)</b>	80.0±0.89	82.0±1.17	83.0±1.51
<b>SGOT(IU/L)</b>	52.16±0.75	54.42±0.84	54.07±0.63
<b>SGPT(IU/L)</b>	25.01±0.16	23.41±0.29	24.26±1.60
<b>Total protein(g/dl)</b>	6.24±0.10	6.30±0.10	6.24±0.10
<b>Albumin(g/dl)</b>	3.04±0.04	3.04±0.04	3.06±0.04
<b>Globulin(g/dl)</b>	5.023±0.03	5.29±0.01	5.15±0.04

Values are mean of 10 animals ± S.E.M (Dunnet's test)\* p<0.05 ;\*\*p<0.01.N=10

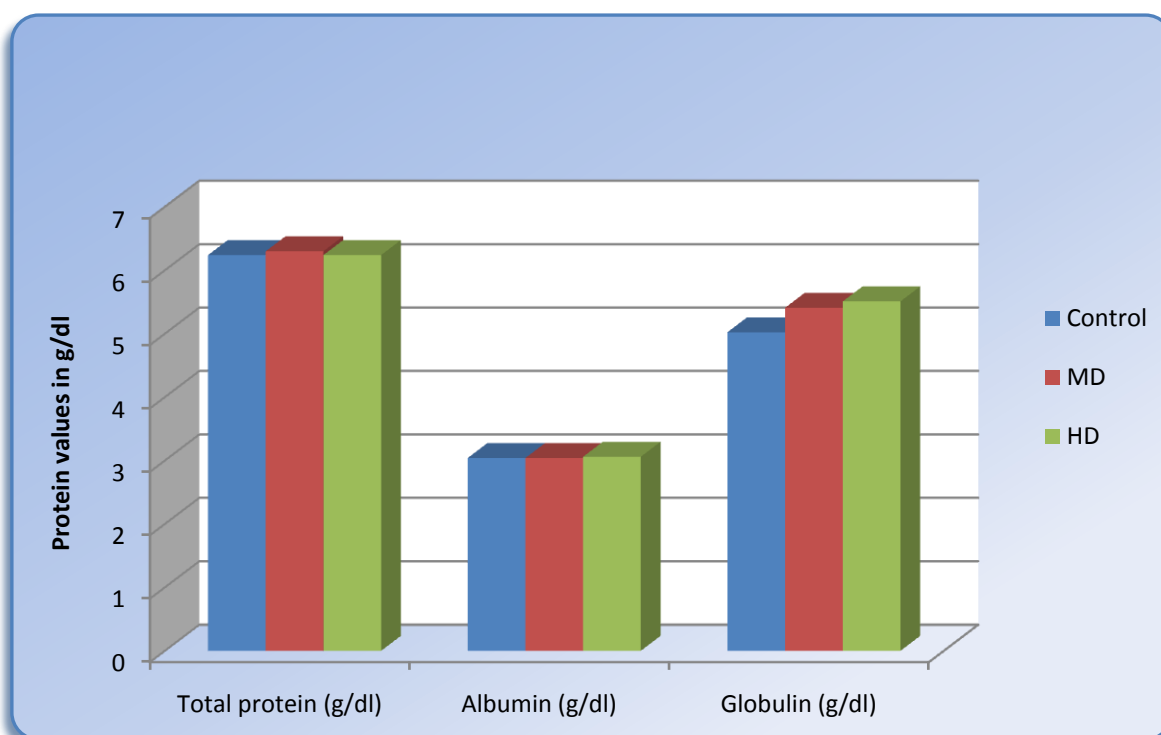
**Chart 4 The mean value of Total Bilirubin, Direct bilirubin and indirect bilirubin of control and treated groups of wistar albino rats exposed to *Indhuppu bhavanai***



**Chart 5** The mean value of ALP,SGOT and SGPT of control and treated groups of wistar albino rats exposed to *Indhuppu bhavanai* for 28 days



**Chart 6** The mean value of Albumin, globulin and total protein of control and treated groups of wistar albino rats exposed to *Indhuppu bhavanai* for 28 days

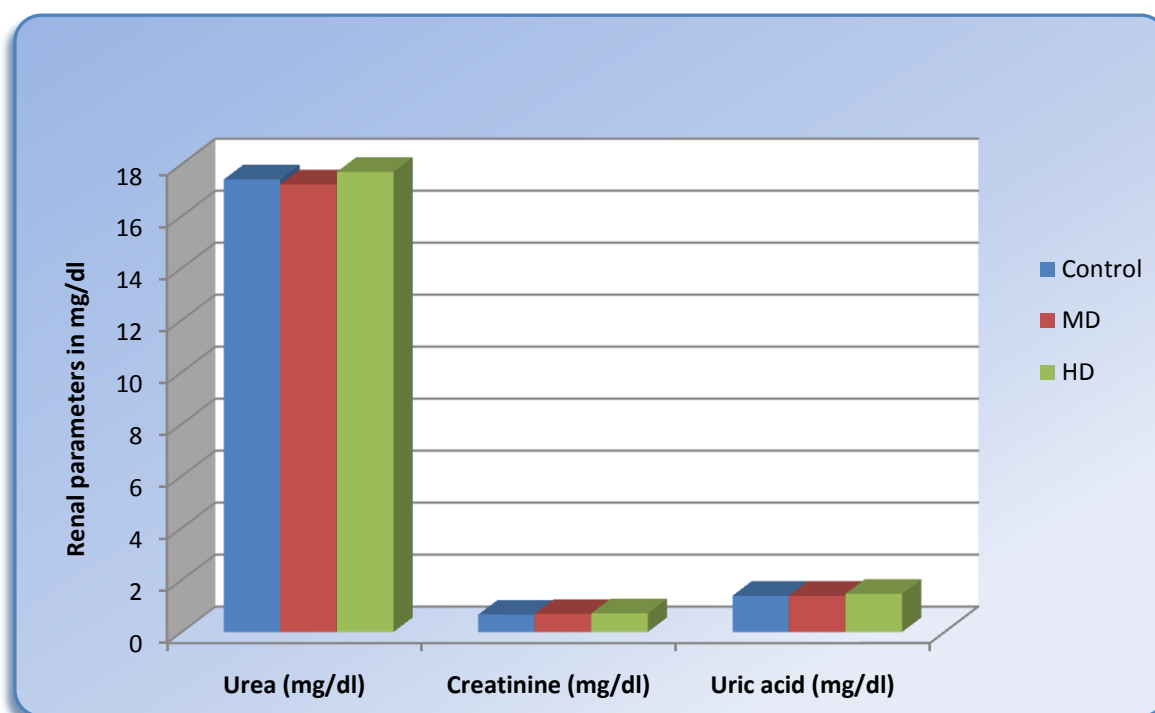


**Table 14 Effect of *Indhuppu bhavanai* on Renal function test**

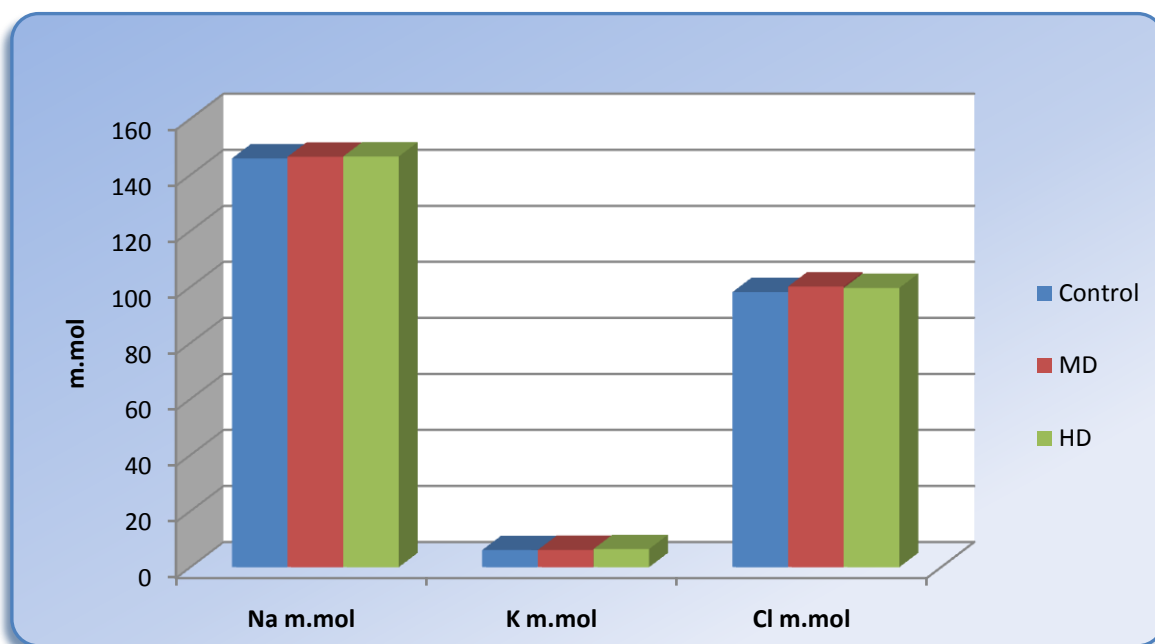
Dose (mg/kg)	Control	MD dose	HD dose
Urea(mg/dl)	17.42±0.10	17.23±0.11	17.71±0.11
Creatinine(mg/dl)	0.67±0.01	0.70±0.02	0.72±0.02
Uric acid(mg/dl)	1.4±0.02	1.4±0.02	1.47±0.04
Na m.mol	145.91±0.55	146.50±0.55	146.66±0.52
K m.mol	6.11±0.01	6.16±0.02	6.46±0.20
Cl m.mol	98.20±0.10	100.20±0.10	99.68±1.10

Values are mean of 10 animals ± S.E.M (Dunnet's test) \*p<0.05 ;\*\*p<0.01.N=10

**Chart 7 The mean value of Urea, Creatinine and uric acid of control and treated groups of wistar albino rats exposed to *Indhuppu bhavanai* for 28 days**



**Chart 8** The mean value of sodium, potassium and chloride levels of control and treated groups of wistar albino rats exposed to *Indhuppu bhavana* for 28 days



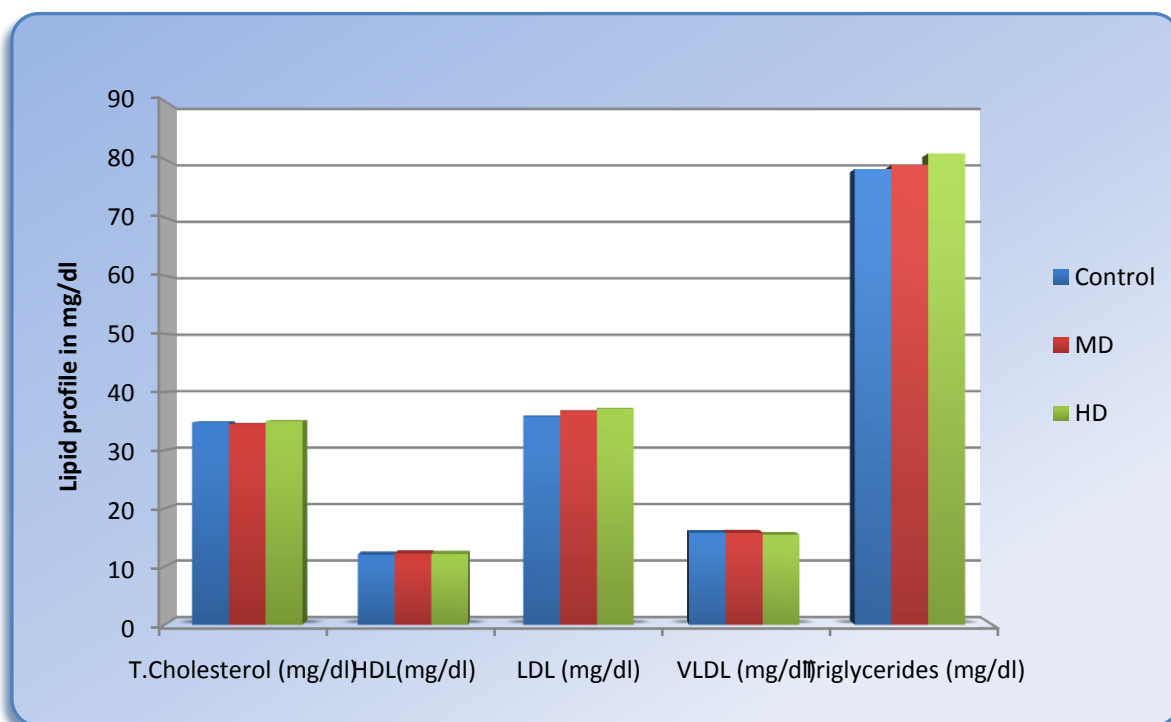
**Table 15: Lipid Profile**

Parameters	Control	MD dose	HD dose
Total cholesterol (mg/kg)	34.86±0.39	34.50±0.38	35.01±0.35
HDL(mg/dl)	12.05±0.02	12.2±0.05	12.17±0.09
LDL(mg/dl)	35.83±0.42	36.73±0.36	37.18±0.70
VLDL (mg/dl)	15.80±0.03	15.81±0.02	15.75±0.04
Triglycerides(mg/kg)	78.33±0.52	79.0±1.26	81 ±0.82
TC/HDL ratio (g/dl)	3.41±0.04	3.36±0.04	3.22±0.03
Blood glucose (mg/dl)	121.90±0.66	121.84±0.82	121.98±0.36

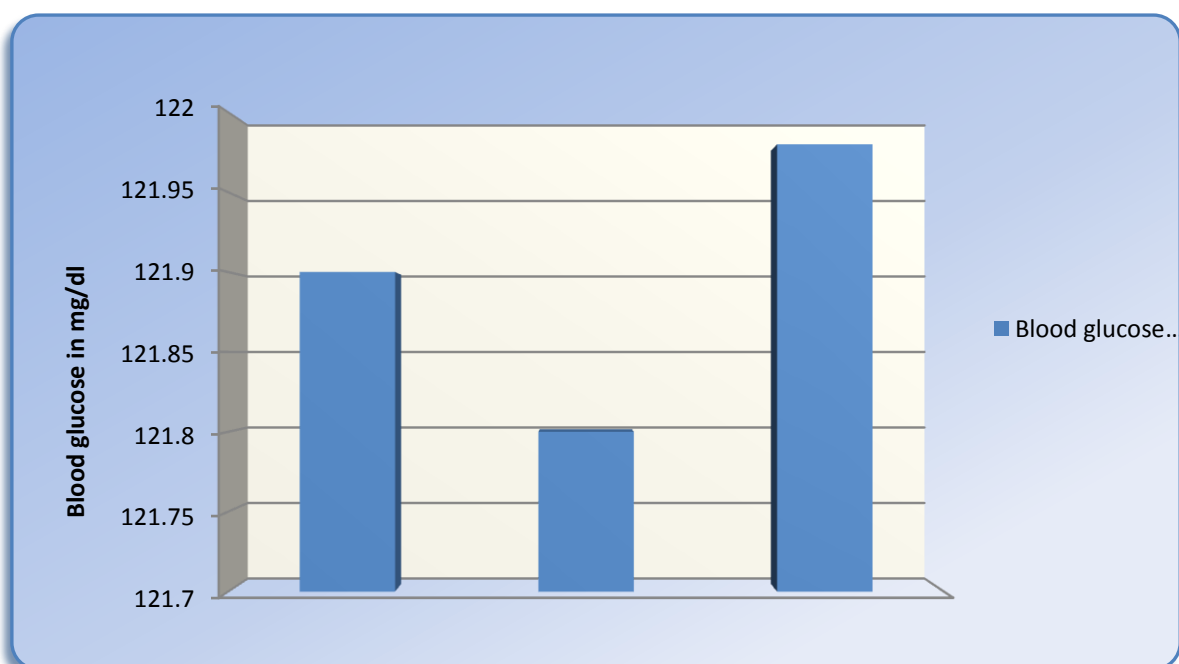
Values are mean of 10 animals ± S.E.M (Dunnet's test) \*p<0.05 ;\*\*p<0.01.N=10



**Chart 9** The mean value of Sr.TC, Sr.TG, HDL,LDL,VLDL of control and treated groups of wistar albino rats exposed to *Indhuppu bhavanai* for 28 days



**Chart 10** The mean value of Blood sugar of control and treated groups of wistar albino rats exposed *Indhuppu bhavanai* for 28 days



**Table 16: Urine Analysis of 28 Days Repeated oral toxicity study of *Indhuppu bhavanai* in wistar albino rats**

Parameters	Control	MD dose	HD dose
<b>Transparency</b>	Clear	Slightly turbid	Slightly turbid
<b>Specific gravity</b>	1.010	1.010	1.010
<b>PH</b>	>7.2	>7.2	>7.2
<b>Protein</b>	Nil	Nil	Nil
<b>Glucose</b>	Nil	Nil	Nil
<b>Bilirubin</b>	-ve	-ve	-ve
<b>Ketones</b>	-ve	-ve	-ve
<b>Blood</b>	Absent	Absent	Absent
<b>Urobilinogen</b>	Normal	normal	normal
<b>Pus cells</b>	0-cells/HPF	0-cells/HPF	1-cells/HPF
<b>RBC</b>	Nil	Nil	1-cells/HPF
<b>Epithelial cells</b>	Nil	1-cells/HPF	Nil
<b>Crystals</b>	Nil	Nil	Nil
<b>Casts</b>	Nil	Nil	Nil
<b>Others</b>	Bacteria seen	Bacteria seen	Bacteria seen
<b>Colour</b>	Yellow	Yellow	Yellow

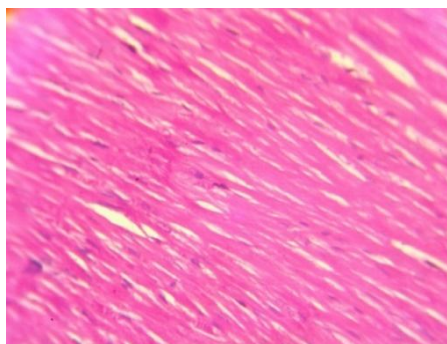
**Table 17:Effect of oral administration of *Indhuppu Bhavanai* on organ weight**

Dose (mg/kg)	Control	MD dose	HD dose
<b>Liver(g)</b>	4.39±0.07	4.51±0.01	4.54±0.03
<b>Heart (g)</b>	0.39±0.04	0.39±0.03	0.39±0.02
<b>Lung(g)</b>	1.30±0.24	1.30±0.20	1.30±0.20
<b>Spleen (g)</b>	0.48±0.16	0.50±0.18	0.50±0.22
<b>Ovary (g)</b>	1.47±0.01	1.49±0.02	1.49±0.02
<b>Testes(g)</b>	1.20±0.10	1.22±0.22	1.22±0.28
<b>Brain(g)</b>	1.34±0.02	1.36±0.02	1.37±0.01
<b>Kidney(g)</b>	0.60±0.04	0.62±0.04	0.62±0.04
<b>Stomach(g)</b>	1.36±0.03	1.38±0.02	1.38±0.02

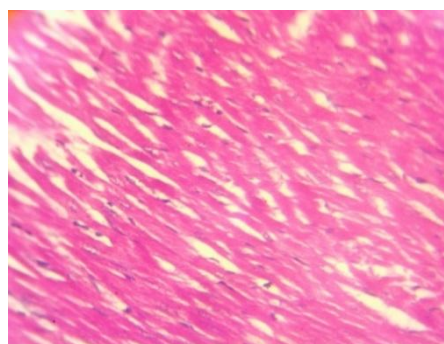
Values are mean of 10 animals ± S.E.M (Dunnet's test) \*p<0.05 ;\*\*p<0.01.N=10

**HISTOPATHOLOGICAL OF VARIOUS ORGANS AFTER THE REPEATED  
DOSE 28 DAY ORAL TOXICITY STUDY OF *INDHUPPU BHAVANAI* IN  
WISTAR ALBINO RATS**

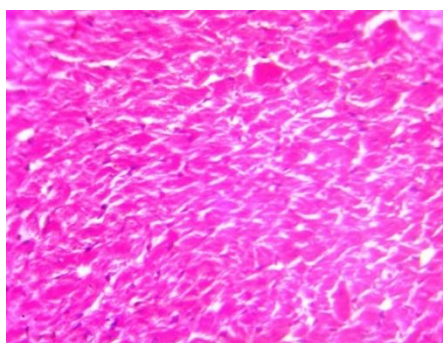
**Heart**



**a. Control**



**b. Mid dose group**

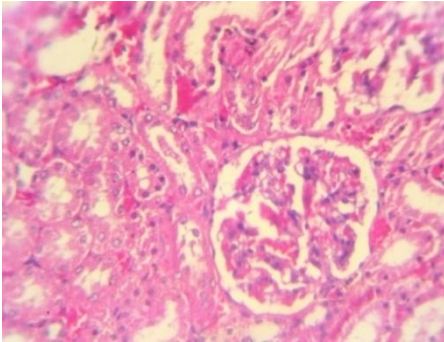


**c. High dose group**

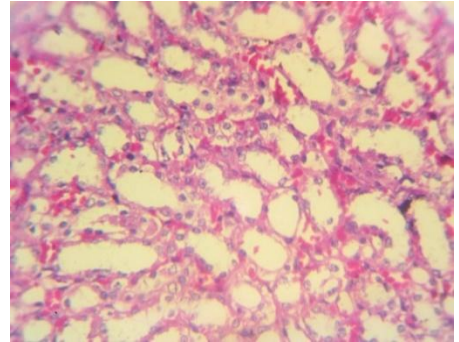
**Observations**

- a.**Section of the heart showed normal muscle fibres with acidophilic cytoplasm and centrally located nuclei.
- b.** Section of the heart showed normal muscle fibres with acidophilic cytoplasm and centrally located nuclei
- c.** Section of the heart showed normal muscle fibres with acidophilic cytoplasm and centrally located nuclei

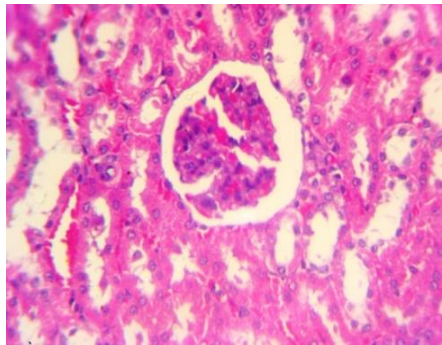
## Kidney



**a. Control**



**b. Mid dose**



**C. High dose**

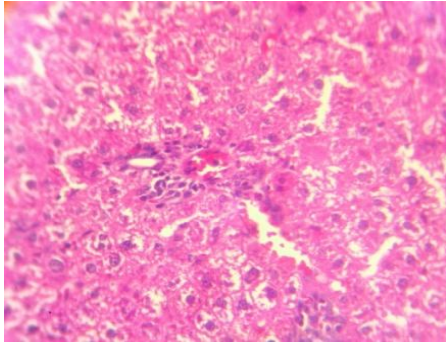
### Observations

a. Section of kidney from control animals showed normal size of glomeruli with normal tubules.

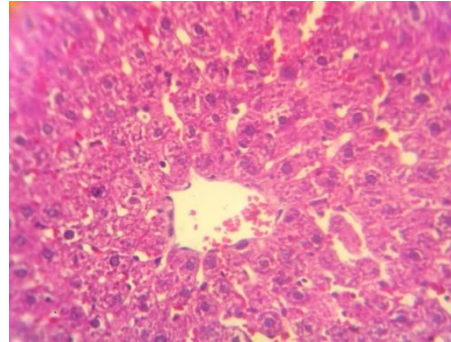
b. Section of kidney showed normal glomeruli and there is no necrosis of tubular epithelium in the kidney.

c. Section of kidney showed normal glomeruli and there is no necrosis of tubular epithelium in the kidney

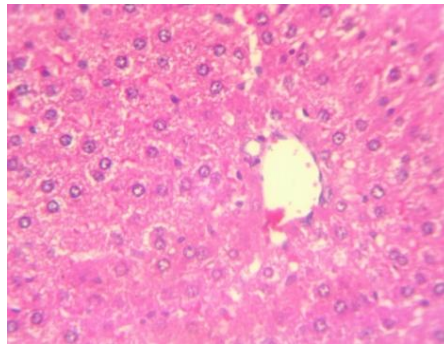
## Liver



**a. Control**



**b. Mid dose**



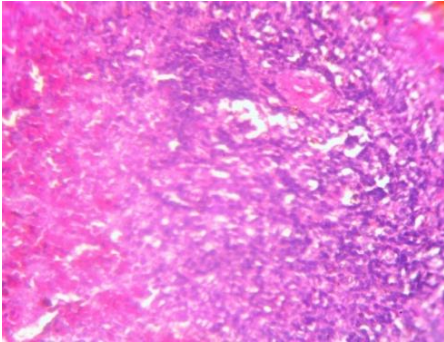
**c. High dose**

### Observations

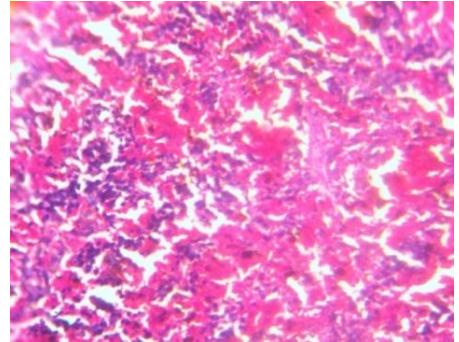
- a. Section of liver from control animals showed no degeneration of hepatocytes, focal steatosis, congestion of central vein and inflammation of portal tract .
- b. Section of liver showed no degeneration of hepatocytes, focal steatosis, congestion of central vein and inflammation of portal tract
- c. Section of liver showed no degeneration of hepatocytes, focal steatosis, congestion of central vein and inflammation of portal tract



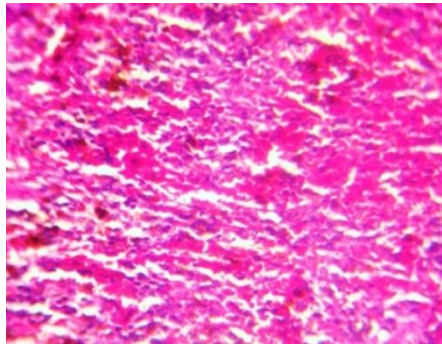
## Spleen



**a. Control**



**b. Mid dose**

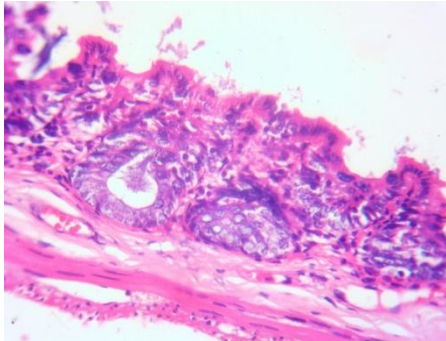


**c. High dose**

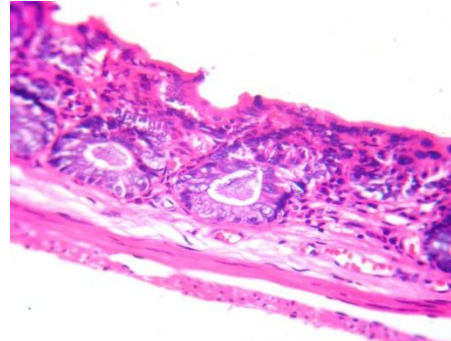
### Observations

- a. Section of spleen from control animal showed normal granular hemosiderin pigment predominantly within macrophages in the red pulp.
- b. Section of spleen showed normal granular hemosiderin pigment predominantly within macrophages in the red pulp with normal structure.
- c. Section of spleen showed normal granular hemosiderin pigment predominantly within macrophages in the red pulp with normal structure

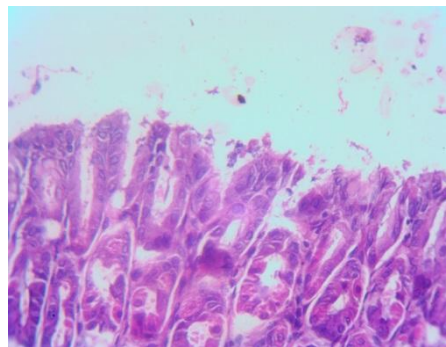
## **Stomach**



a. Control



b. Mid dose



C. High dose

### **Observation**

- a. Section of stomach shows gastric mucosa lined by tall columnar cells that detected no abnormality.
- b. Section of stomach shows gastric mucosa lined by tall columnar cells that detected no abnormality
- c. Section of stomach shows gastric mucosa lined by tall columnar cells that detected no abnormality.



## **RESULTS OF REPEATED DOSE 28 DAYS ORAL TOXICITY STUDY**

Repeated dose 28 days oral toxicity of *Indhuppu bhavanai* on rats were conducted. All animals from the treated dose survived throughout the dosing period of 28 days. Various parameters were studied and the interpretation of the study result was discussed below.

**Clinical signs:** No abnormal behavioural signs were observed during the study period.

### **Mortality**

The test drug “*Indhuppu bhavanai*” did not cause any mortality in mid and high dose levels and were considered as safe dose levels.

### **Body weight:**

The result of the body weight of rats exposed to control and the trial drug of different dose groups exhibited over all mild weight gain throughout the dosing period of 28 days. The quantity of food taken by the animals from different dose groups and the control is comparably normal.

### **Haematological investigation interpretation**

The haematological investigation results of the rats conducted on 28th day after the repeated dose of the drug revealed the values of different parameters. The increase and decrease in the values obtained were all within the normal biological and laboratory limits.

### **Biochemical investigation interpretation**

The biochemical investigations were conducted on 28th day and the result was produced. The results revealed there was no significant changes in the values of different parameters with that of the control. All the values were within the normal biological and laboratory limits.

### **Histopathology interpretation**

Histopathological study of the organ such as heart, kidney, liver, spleen and stomach were normal in control and all test groups.

**Table 18 : Body wt of wistar albino rats exposed to *Indhuppu bhavanai* for 90 days**

DAYS	Weight(gms)/Days				P value (p) *
	Control	Low dose	Mid dose	High dose	
1	161.6±33.68	148.1 ± 21.11	156.6± 13.57	159.5± 28.75	NS
15	172.8 ± 28.87	162.5 ± 21.71	166.7 ± 29.01	166.8 ± 32.13	NS
30	181.8 ± 28.31	175.71 ± 14.88	181.8 ± 32.11	181± 28.94	NS
45	204.5± 27.73	194.5± 29.76	204.7± 19.75	208.3± 22.75	NS
60	218.6±33.68	214.6±23.68	226.6±33.68	215.6±23.78	NS
75	236.8 ± 26.85	226.8 ± 28.87	241.8± 28.87	224.8 ±26.87	NS
90	248.5± 27.32	239.5± 27.68	251.4± 27.32	236.3± 36.32	NS

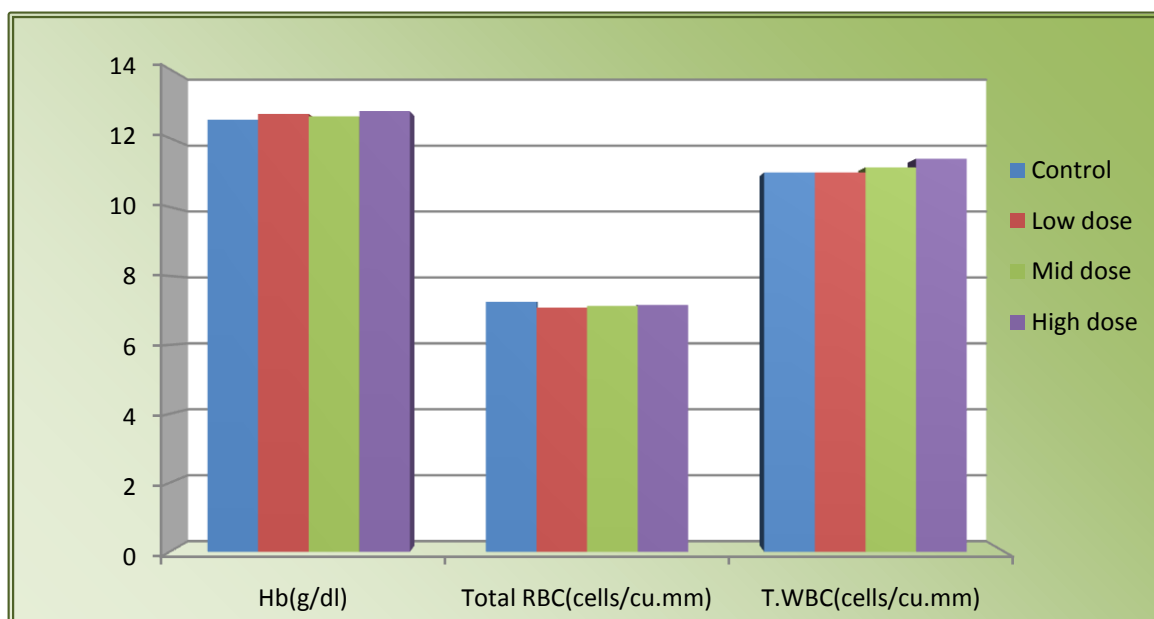
NS- Not Significant, \*\*( $p < 0.01$ ), \*( $p < 0.05$ ), n = 6 values are mean ± S.D (One way ANOVA followed by Dunnett's test)

**Table 19: Haematological parameters of Wistar albino rats group exposed to *Indhuppu bhavanai* for 90 days**

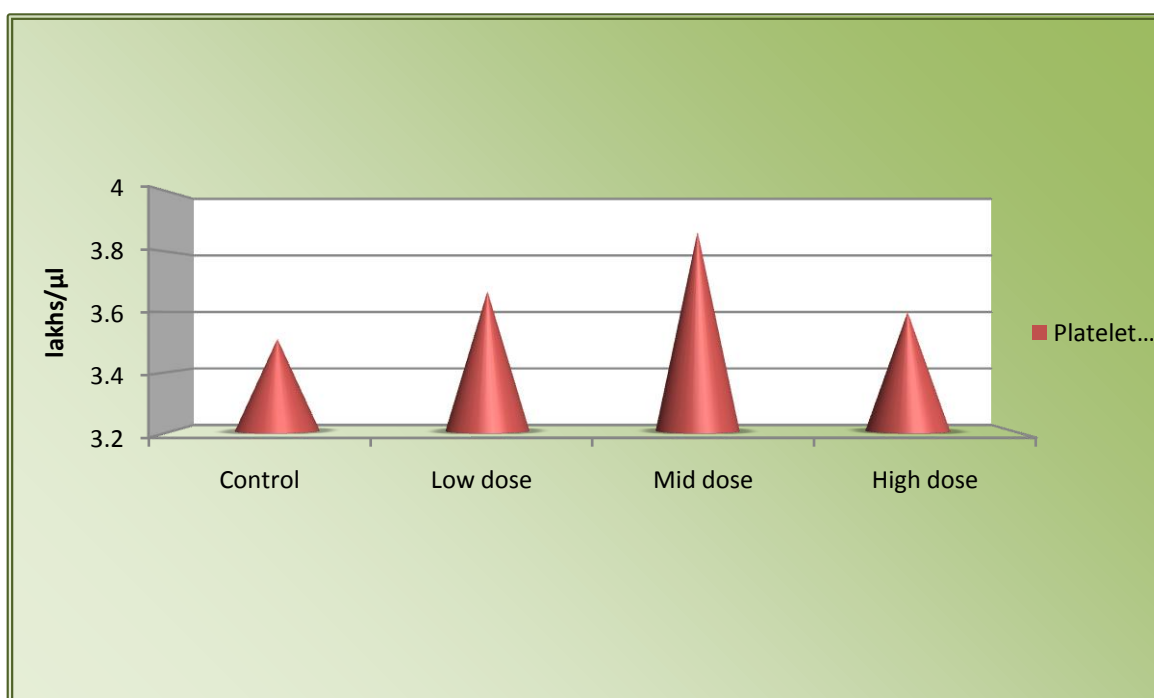
Category	Control	Low dose	Mid dose	High dose	P value (p)*
<b>Haemoglobin(g/dl)</b>	12.53±0.31	12.7±0.59	12.63±0.45	12.78±0.67	N.S
<b>Total WBC (cells/cu.mm)</b>	11.2±4.02	11±0.54	11.15±5.12	11.4±6.37	N.S
<b>Platelets lkhs/μl</b>	351±.21	367±25	387.05±26	36±33	N.S
<b>Total RBC (cells/cu.mm)</b>	7.25 ± 0.3	7.08±0.3	7.13 ± 0.2	7.16±0.3	N.S
<b>PCV%</b>	37.59±0.95	38.29±0.18	37.9±1.34	38.36±2.05	N.S
<b>MCHC g/dl</b>	30±3.03	33.66±1.86	31±1.22	30.5±0.07	N.S
<b>MCV%ft</b>	92.16±2.62	91.3±3.75	92.3±1.98	91.5±1.89	N.S
<b>MCH pg</b>	30± 3.03	32.75±1.86	31±1.22	30.5±0.07	N.S

N.S- Not Significant, \*\*( $p < 0.01$ ), \*( $p < 0.05$ ), n = 6 values are mean ± S.D (One way ANOVA followed by Dunnett's test)

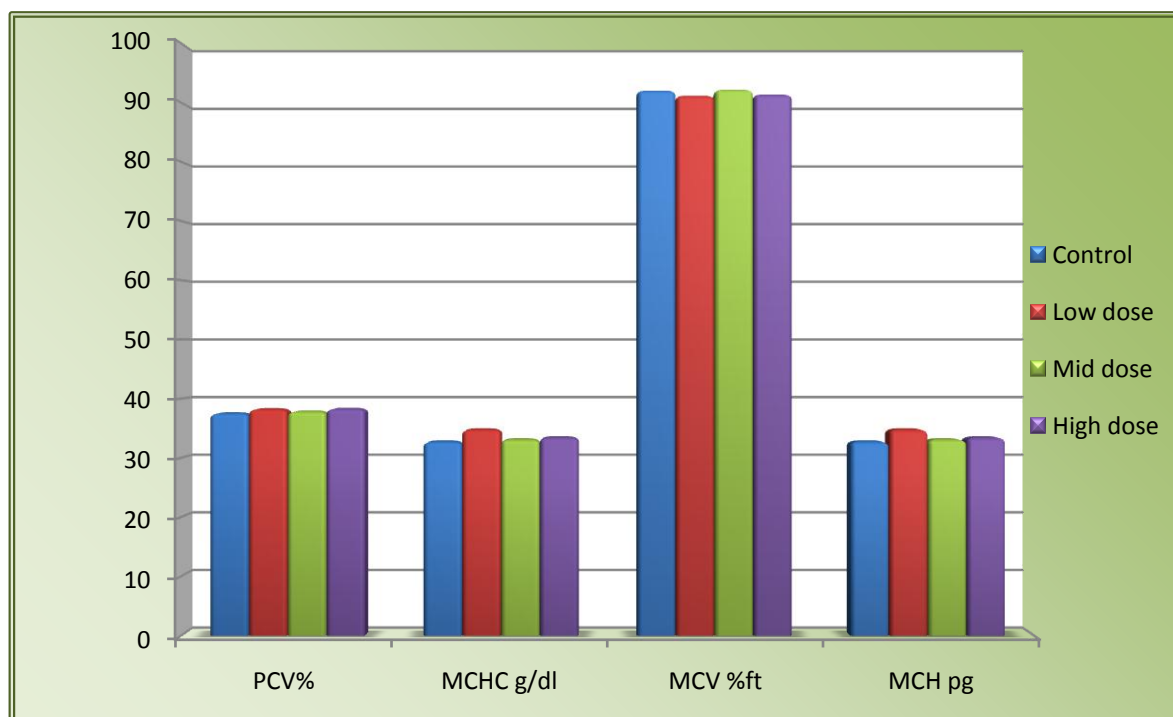
**Chart 11: The mean value of HB, Total RBC, Total WBC of control and treated groups of wistar albino rats exposed *Indhuppu bhavanai* for 90 days**



**Chart 12: The mean value of Platelets of control and treated groups of wistar albino rats exposed *Indhuppu bhavanai* for 90 days**



**Chart 13:**The mean value of PCV, MCHC, MCV, MCH of control and treated groups of wistar albino rats exposed *Indhuppu bhavanai* for 90 days

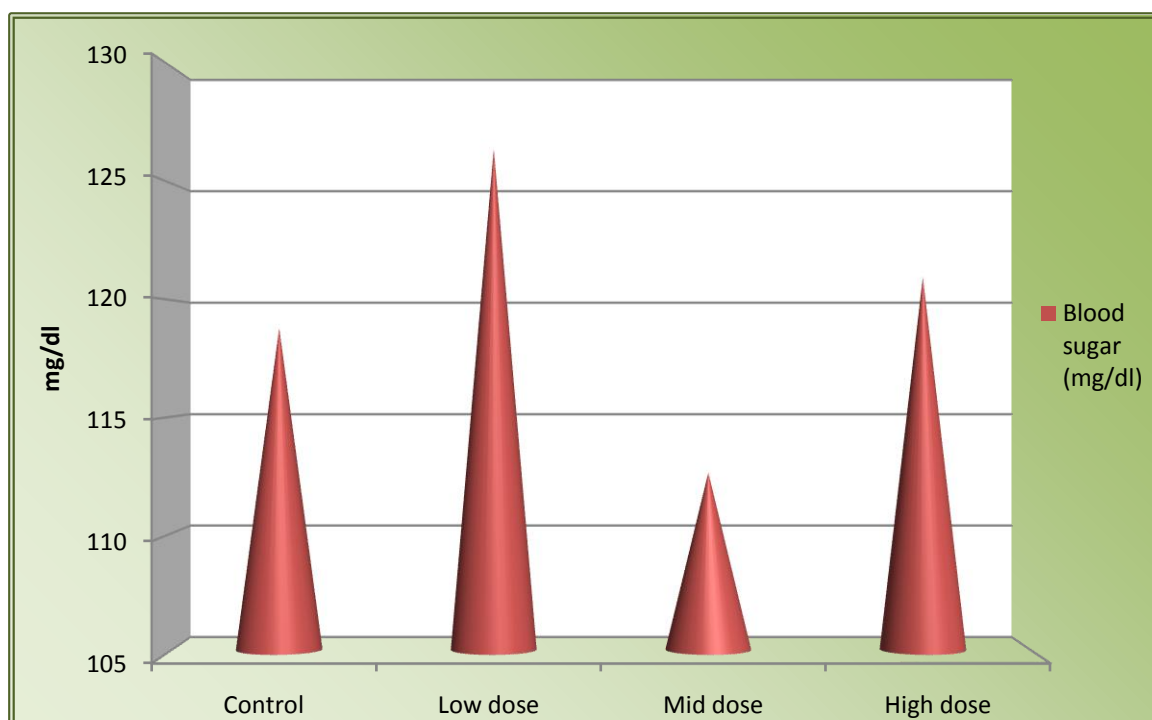


**Table 20:** Blood sugar test of Wistar albino rats group exposed to *Indhuppu bhavanai* for 90 days

PARAMETERS	CONTROL	LOW DOSE	MID DOSE	HIGH DOSE	P Value (p)*
Bl.sugar (mg/dl)	118.30±8	126.5±6.28	112.6±16.1	121±20.9	N.S.

N.S- Not Significant, \*\*( $p < 0.01$ ), \*( $p < 0.05$ ),  $n = 6$  values are mean  $\pm$  S.D (One way ANOVA followed by Dunnett's test)

**Chart 14:**The mean value of Blood sugar of control and treated groups of wistar albino rats *Indhuppu bhavanai* for 90 days

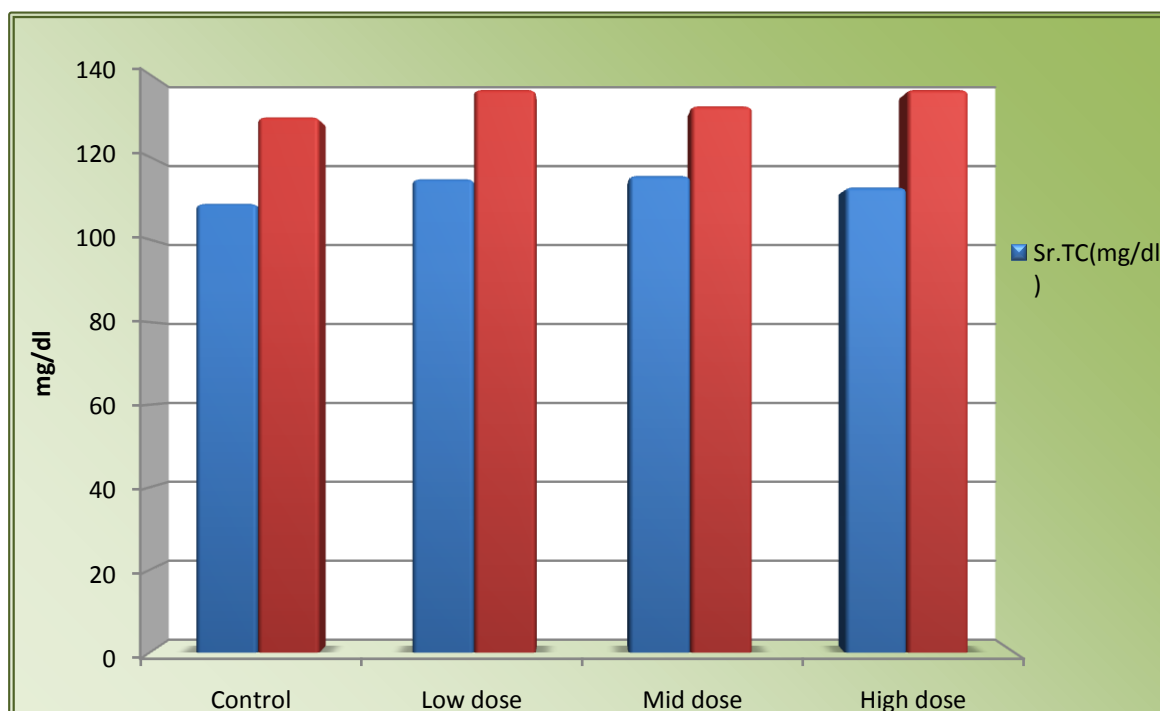


**Table 21:** Lipid profile test of Wistar albino rats group exposed to *Indhuppu bhavanai* for 90 days

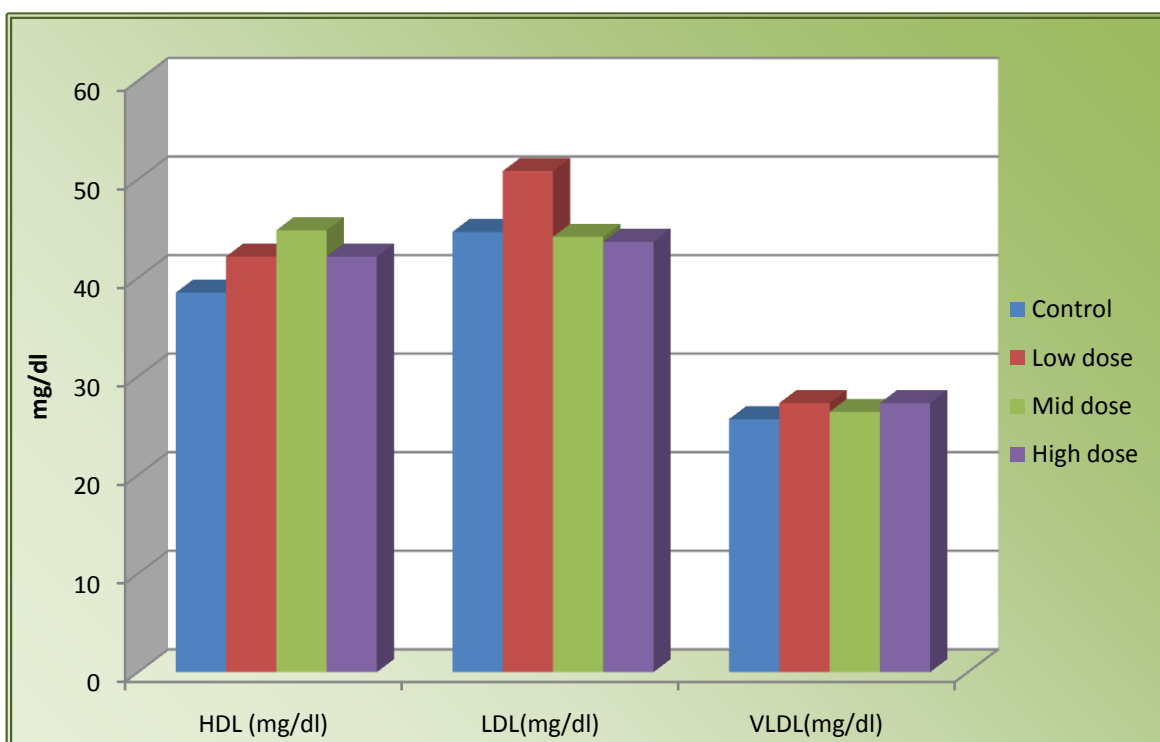
PARAMETERS	CONTROL	LOW DOSE	MID DOSE	HIGH DOSE	P Value (p)*
Sr.TC(mg/dl)	108.±8.3	114.83±12.93	115.6±8.28	112.83±6.76	N.S
Sr.TG(mg/dl)	129.83±2.4	136.55±9.35	4132.5±9.73	136.5±9.31	N.S
HDL (mg/dl)	38.5±1.64	41.16±4.40	42.69±2.04	42.83±4.02	N.S
LDL(mg/dl)	44.66±9.30	50.83±12.54	44.16±8.68	43.66±7.33	N.S
VLDL(mg/dl)	25.66±1.25	27.3±1.87	26.4±2.01	27.3±1.86	N.S

N.S- Not Significant, \*\*( $p < 0.01$ ), \*( $p < 0.05$ ),  $n = 6$  values are mean  $\pm$  S.D (One way ANOVA followed by Dunnett's test)

**Chart 15:**The mean value of Sr.TC, Sr.TG of control and treated groups of wistar albino rats exposed *Indhuppu bhavana* for 90 days



**Chart 16:**The mean value of HDL, LDL, VLDL of control and treated groups of wistar albino rats exposed *Indhuppu bhavana* for 90 days

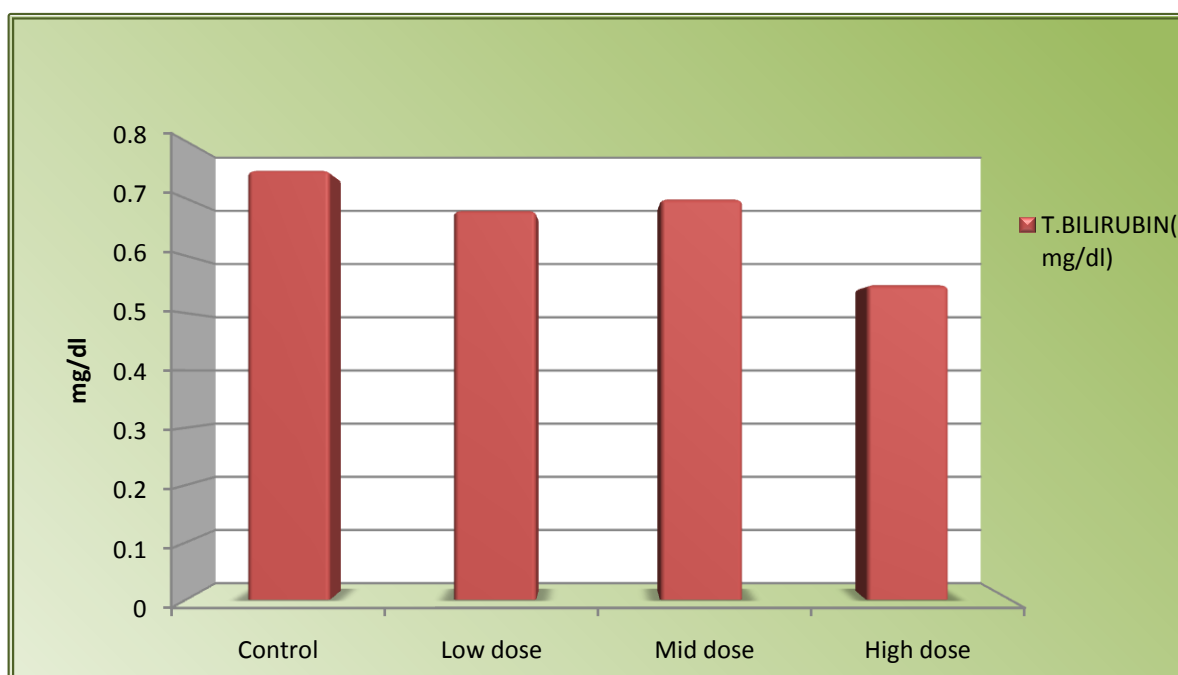


**Table 22: Liver Function Test of Wistar albino rats group *Indhuppu bhavanai* for 90 days**

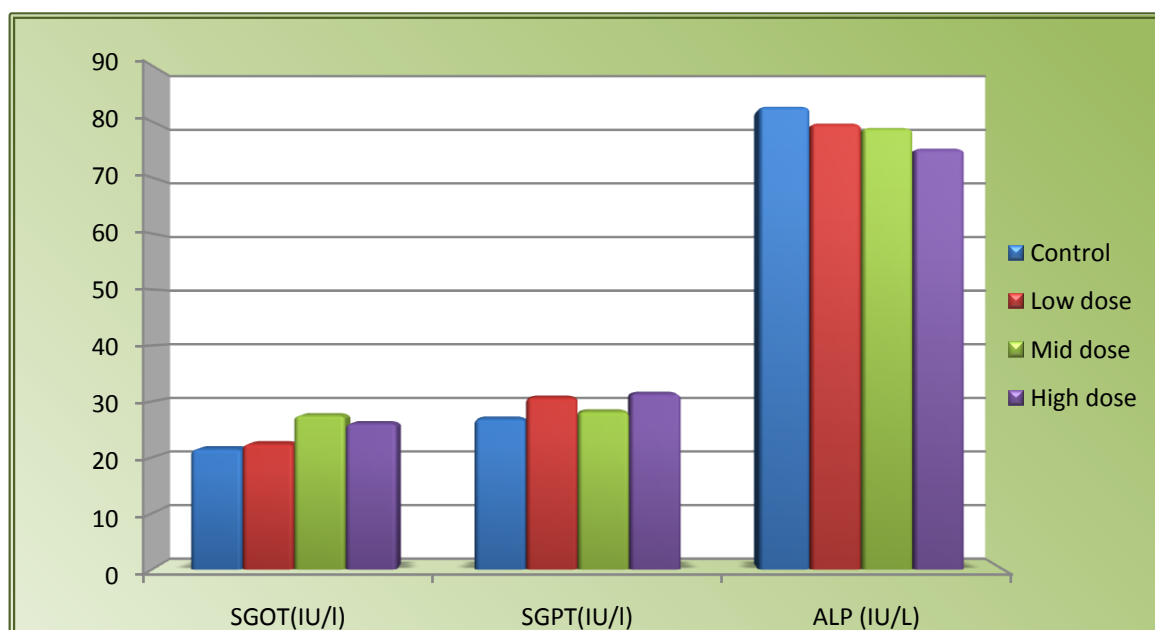
PARAMETERS	CONTROL	LOW DOSE	MID DOSE	HIGH DOSE	P Value (p)*
<b>T.BILIRUBIN(mg/dl)</b>	0.86±0.04	0.79±0.1	0.75±0.1	0.80±0.13	N.S
<b>SGOT(IU/L)</b>	21.6±5.08	22.5±9.97	27.6±9.09	26.15±2.04	N.S
<b>SGPT(IU/L)</b>	27±5.9	30.8±5.45	28.5±3.72	31.57±10.9	N.S
<b>ALP (IU/L)</b>	82.83±17.56	79.8±12.09	79±15.7	75.3±17.84	N.S

NS- Not Significant, \*\*( $p < 0.01$ ), \* ( $p < 0.05$ ), n = 6 values are mean  $\pm$  S.D (One way ANOVA followed by Dunnett's test)

**Chart 17: - The mean value of Bilirubin of control and treated groups of wistar albino rats *Indhuppu bhavanai* for 90 days**



**Chart 18:**The mean value of SGOT, SGPT, ALP of control and treated groups of wistar albino rats exposed to *Indhuppu bhavana* for 90 days



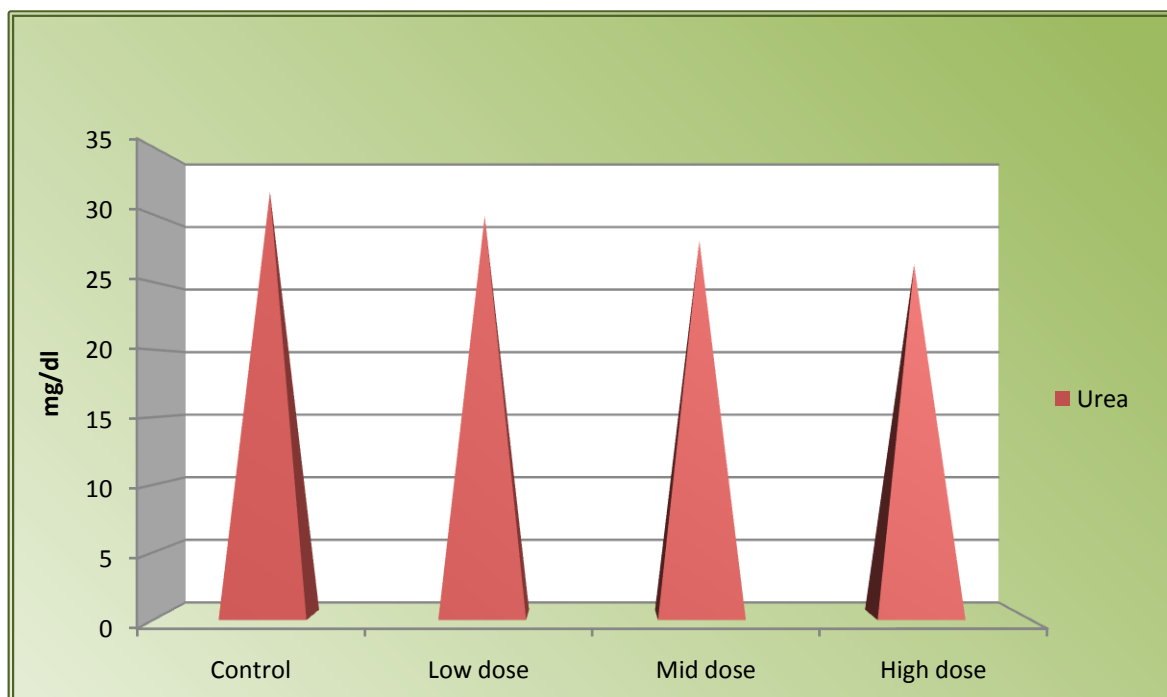
**Table 23 Renal Function Test of Wistar albino rats group *Indhuppu bhavana* for 90 days**

Parameters	Control	Low dose	Mid dose	High dose
Urea (mg/dl)	32	30.16	28.3	26.5
Creatinine (mg/dl)	0.48	0.5	0.51	0.53

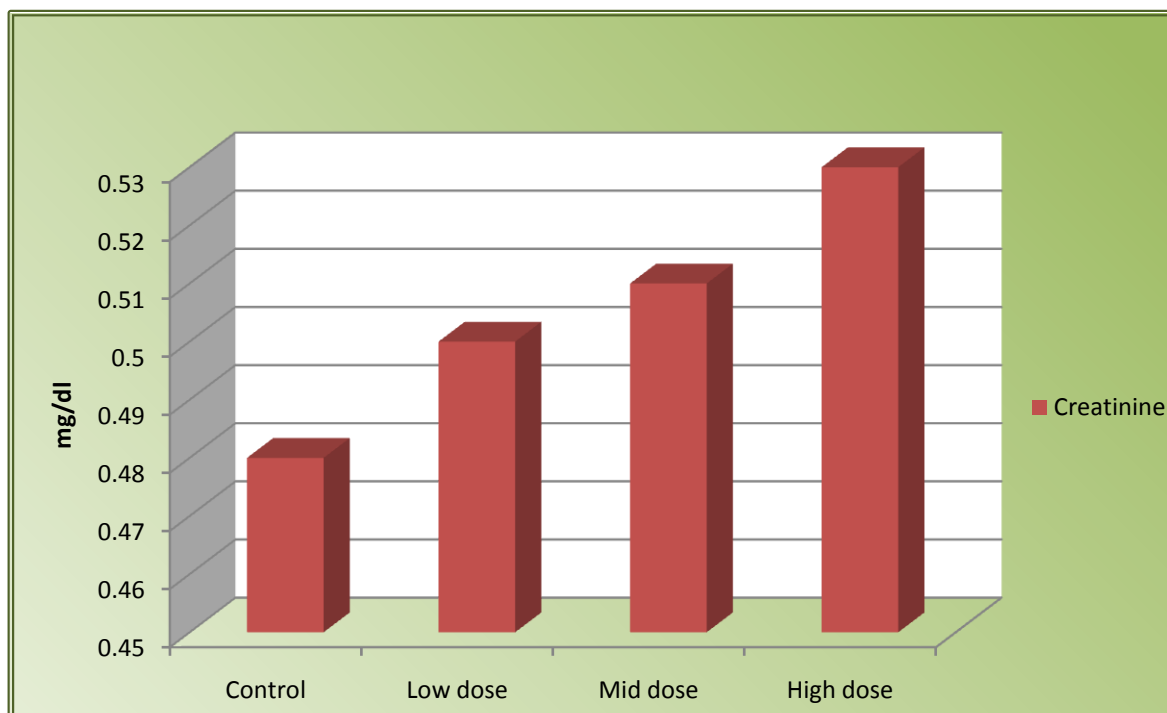
NS- Not Significant, \*\*( $p < 0.01$ ), \* ( $p < 0.05$ ),  $n = 6$  values are mean  $\pm$  S.D (One way ANOVA followed by Dunnett's test)



**Chart 19:**The mean value of Urea of control and treated groups of wistar albino rats exposed to *Indhuppu bhavanai* for 90 days

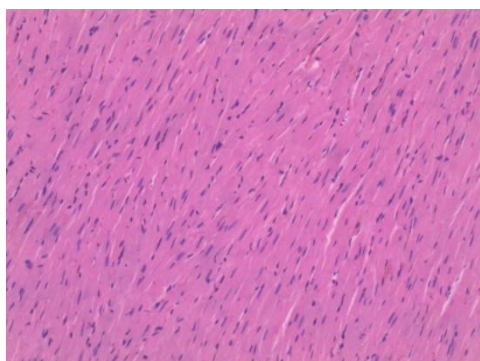


**Chart 20:**The mean value of Creatinine of control and treated groups of wistar albino rats exposed to *Indhuppu bhavanai* for 90 days

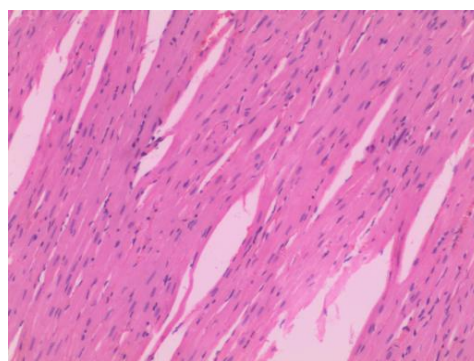


**HISTOPATHOLOGICAL STUDIES OF VARIOUS ORGANS AFTER THE  
REPEATED DOSE 90 DAY ORAL TOXICITY STUDY OF *INDHUPPU*  
*BHAVANAI* IN WISTAR ALBINO RATS**

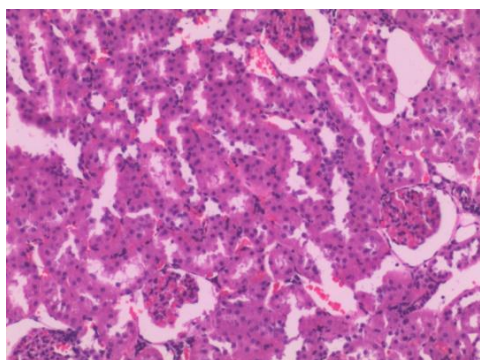
**Control - Heart**



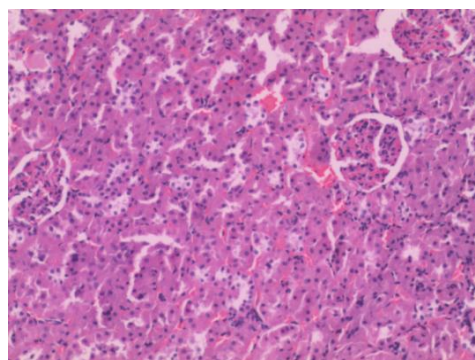
**High dose - Heart**



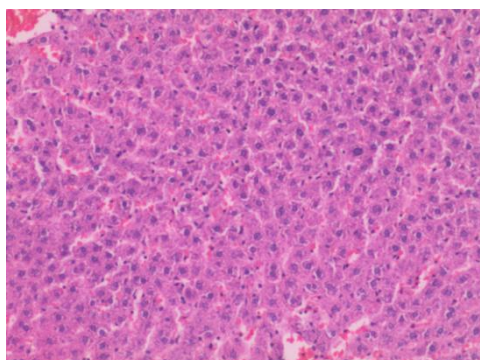
**Control - Kidney**



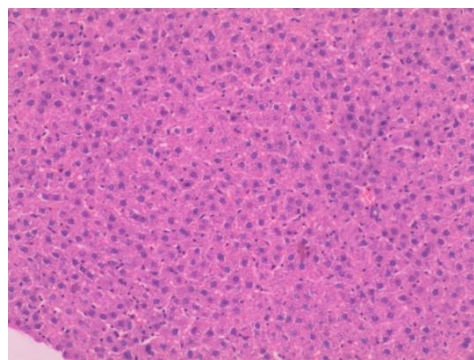
**High dose - Kidney**



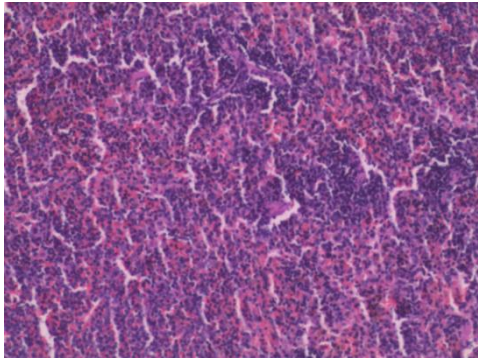
**Control- Liver**



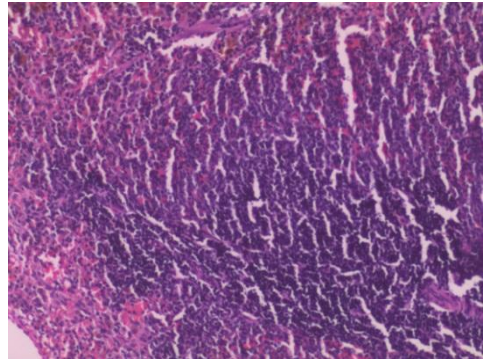
**High dose - Liver**



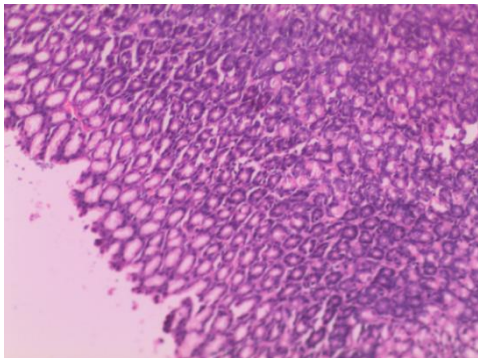
**Control - Spleen**



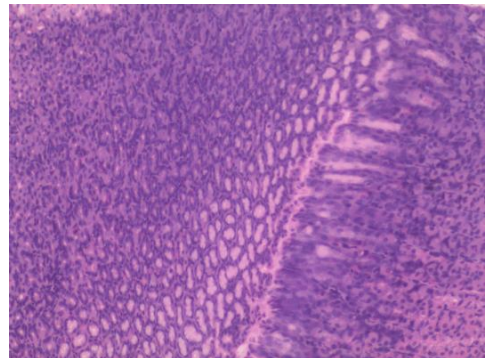
**High dose - Spleen**



**Control - Stomach**



**High dose - Stomach**



**INTERPRETATION FOR HISTOPATHOLOGICAL SLIDES OF *INDHUPPU*  
*BHAVANAI* FOR 90 DAYS**

**Kidney**

- Appearance of glomerular architecture was normal in control and glomeruli showing striking lobular architecture in sample high dose. Cortex and medulla appears normal opening of lumen appears normal
- Increased glomerular permeability was observed.

**Heart**

- Cardiac muscle appears continuous with intact nucleus
- Myocardial tissue appears normal with orderly striated heart muscle fibers and a clear nuclear and muscle bands.

## **Liver**

- Central vein appears prominent with no signs of cellular infiltration
- Hepatocellular architecture, including hepatic sinusoid and hepatic cord was normal

## **Spleen**

- Marginal vascular zone radiated in between red and white pulp
- No signs of immunological activities

## **Stomach**

- Mucosal epithelium appears normal with no signs of ulceration
- Microscopic analysis of stomach of both the sample reveals anatomy of muscular stomach with epithelial layer keratinized stratified squamous epithelium, Lamina propria and Sub-mucosa
- Gastric wall architecture appears normal.

## **RESULTS OF REPEATED DOSE 90 DAYS ORAL TOXICITY STUDY**

Repeated dose 90 days oral toxicity study of *Indhuppu bhavanai* on rats were conducted. All animals from the treated dose survived throughout the dosing period of 90 days. No abnormal behavioural signs were observed during the study period.

The result of the body weight of rats exposed to control and the trial drug of different dose groups exhibited over all mild weight gain throughout the dosing period of 90 days.

The haematological and bio chemical investigations were conducted on 91<sup>st</sup> day after the repeated dose of the drug revealed there is no significant changes in the values of different parameters with that of the control.

Histopathological study of the organ such as heart, kidney, liver, spleen and stomach were normal in control and test groups.

## PHARMACOLOGICAL ACTIVITY OF INDHUPPU BHAVANAI FOR ANTI –ULCER ACTIVITY

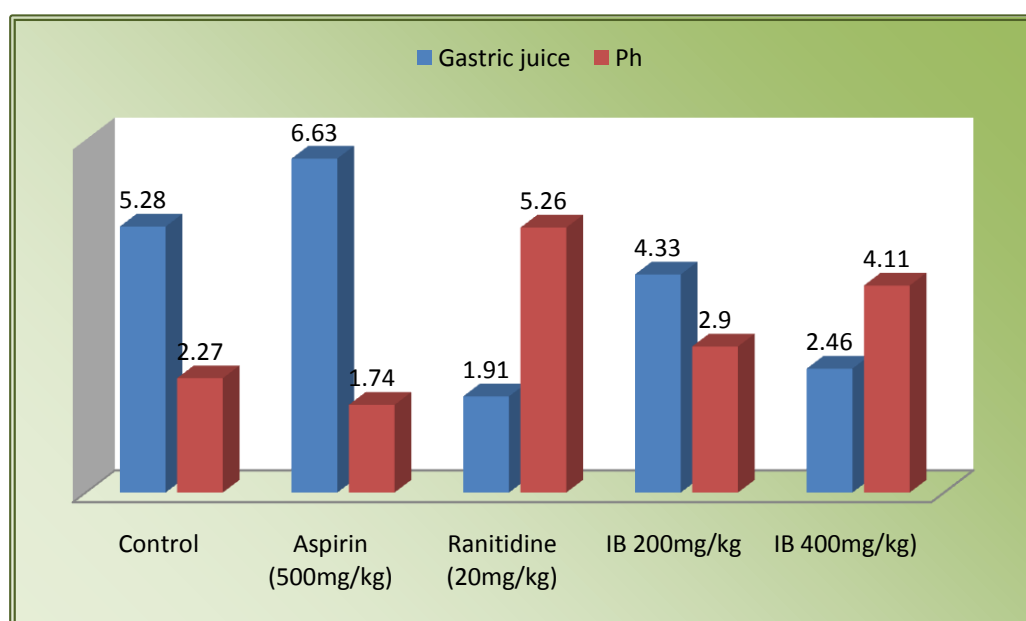
**Table :24** Effect of *Indhuppu bhavanai* (IB) on volume of gastric juice

Group no	Body wt.gms	Treatment	Vol.of gastric juice	Ph
I	170.8 $\pm$ 0.75	Control (distilled water 2ml/kg)	5.28 $\pm$ 0.20	2.27 $\pm$ 0.19
II	172.13 $\pm$ 0.33	Negative control (Aspirin 500mg/kg)	6.63 $\pm$ 0.18	1.74 $\pm$ 0.06
III	171.5 $\pm$ 1.38	Standarddrug ranitidine (20mg/kg)+ Aspirin	1.91 $\pm$ 0.38**	5.26 $\pm$ 0.11**
IV	171.5 $\pm$ 1.4	IB - 200mg/kg + Aspirin	4.33 $\pm$ 0.24*	2.90 $\pm$ 0.48
V	172.2 $\pm$ 0.41	IB - 400mg/kg +Aspirin	2.46 $\pm$ 0.24**	4.11 $\pm$ 0.10**

Effects are statistically significant. \*P<0.05;\*\*P<0.01 (in comparison with standard).

Values are expressed in terms of mean  $\pm$  SEM of 6 rats (ANOVA).

**Chart 21**Effect of *Indhuppu bhavanai* on volume of gastric juice and pH



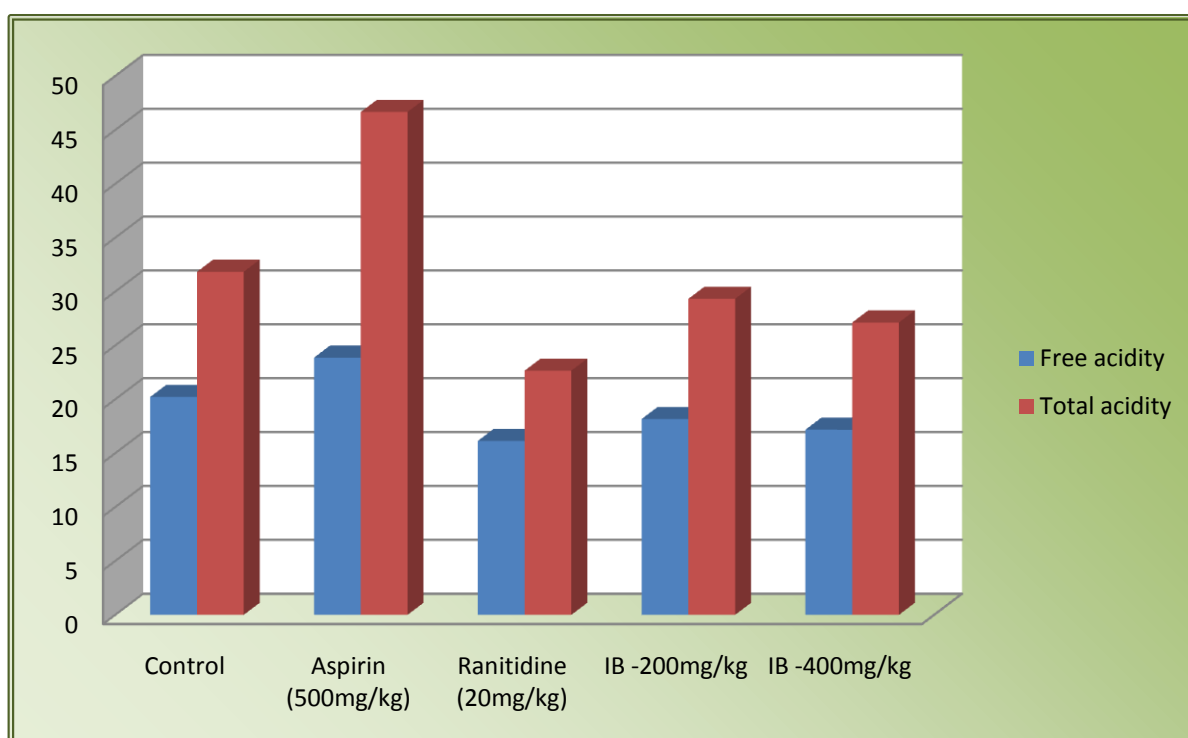


**Table 25 Effect of *Indhuppu bhavanai* on Free acidity and Total acidity**

Group no	Body wt. gms	Treatment	Free acidity	Total acidity
I	170.8 $\pm$ 0.75	Control(distilled water 2ml/kg)	20.22 $\pm$ 0.97	31.84 $\pm$ 2.04
II	172.13 $\pm$ 0.33	Negative control (Aspirin 500mg/kg)	23.87 $\pm$ 0.86	46.67 $\pm$ 1.36
III	171.5 $\pm$ 1.38	Standard drug (Ranitidine- 20 mg/kg) + Aspirin	16.14 $\pm$ 0.19**	22.66 $\pm$ 1.01**
IV	171.5 $\pm$ 1.4	IB -200mg/kg +Aspirin	18.17 $\pm$ 0.09*	29.33 $\pm$ 0.34*
V	172.2 $\pm$ 0.41	IB – 400mg/kg +Aspirin	17.18 $\pm$ 0.14**	27.13 $\pm$ 0.17**

Values are expressed in terms of mean  $\pm$  SEM of 6 rats(ANOVA). Effects are statistically significant \*P<0.05;\*\*P<0.01 ( in comparison with standard)

**Chart :22Effect of *Indhuppu bhavani* on Free acidity and Total acidity**



**Table26 Effect of Indhuppu bhavanai on Ulcer index and Percentage of ulcer protection**

Group no	Body wt.gms	Treatment	Ulcer Index	Percentage of ulcer protection
I	181.3 ± 1.5	Control distilled water 2 ml/kg	6.6 ± 0.26	—
II	182.1 ± 2.0	Negative control(Aspirin 500mg/kg)	7.4 ± 0.50	23.5
III	181.5 ± 0.84	Standard drug-Ranitidine (20mg/kg)+Aspirin	1.25 ± 0.17**	91.5**
IV	181.83 ± 1.6	IB- 200mg/kg+Aspirin	3.45 ± 0.37*	54.5
V	181.3 ± 1.63	IB – 400 mg/kg+ Aspirin	2.21 ± 0.24**	75.5*

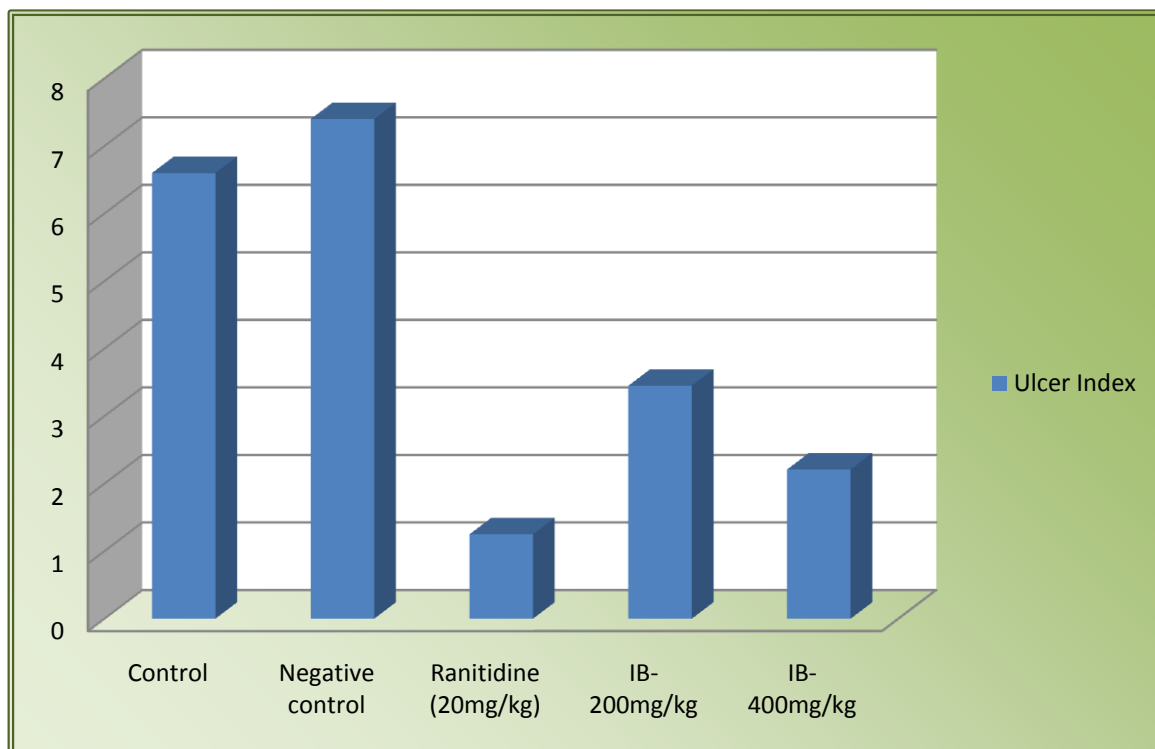
Values are expresses in terms of mean ± SEM of 6 rats (ANOVA).

Percentage protection = ( Control meanulcer index – Test mean ulcer index )/Control meanulcer index X 100.

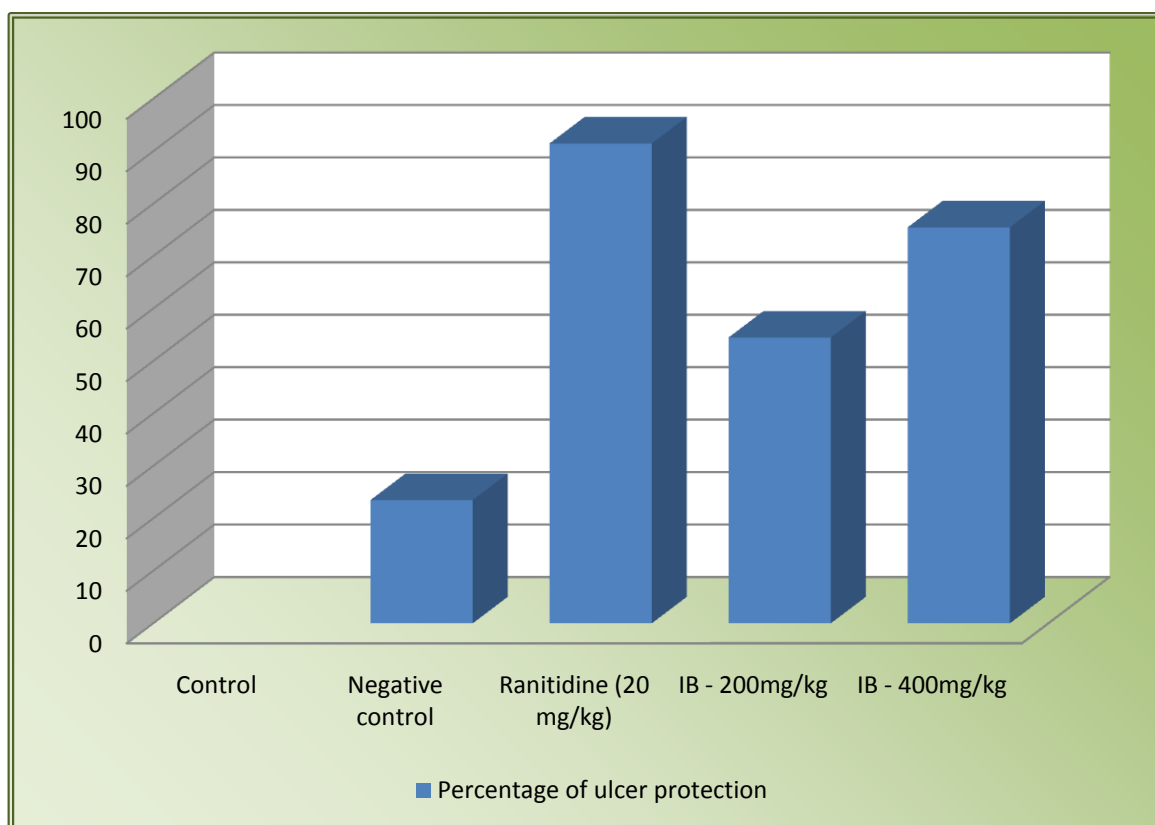
Effects are statistically significant. \*P<0.05;\*\*P,0.01 (in comparison with Standard)

Group I - Control  
Group II - Negative control received aspirin (500mg/kg)  
Group III - Standard drug ranitidine + Aspirin (500mg/kg)  
Group IV - Indhuppu bhavanai 200mg/kg + Aspirin (500mg/kg)  
Group V - Indhuppu bhavanai 400mg/kg + Aspirin (500mg/kg)

**Chart 23** Effect of *Indhuppu bhavanai* on Ulcer index



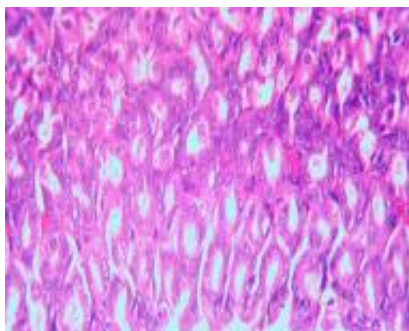
**Chart 24** Effect of *Indhuppu bhavanai* on percentage of ulcer protection



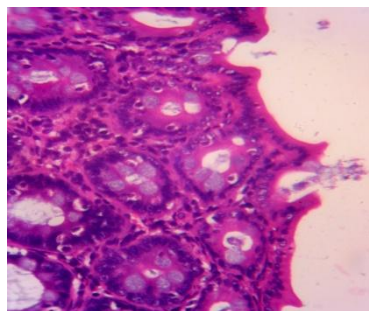


## HISTOPATHOLOGICAL SLIDES OF OPEN EXCISED STOMACH IN ASPIRIN INDUCED GASTRIC LESIONS MODEL

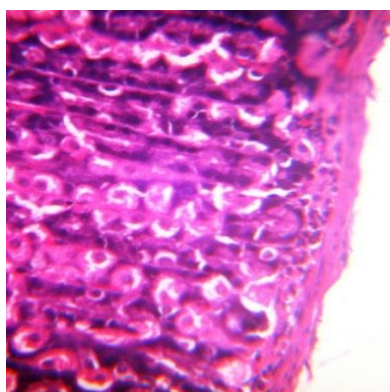
a. Control



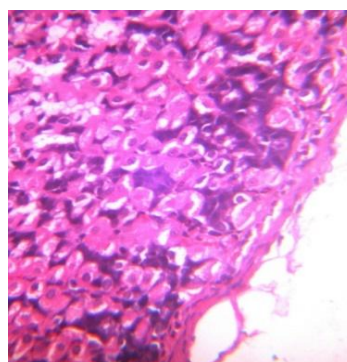
b. Negative control



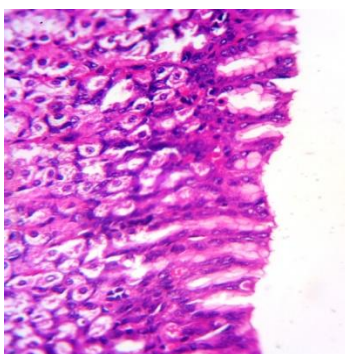
c. Standard drug – Ranitidine



d. IB 200mg/dl



e. IB- 400mg/dl



a. Inhibition of gastric lesions at control

b. Gastric lesions induced by Aspirin (500mg/dl)

c. Absence of gastric lesions in Ranitidine (20mg/kg)

d. Fractional inhibition of gastric lesions at Indhuppu Bhavanai (200mg/kg)

e. Fractional inhibition of gastric lesions at Indhuppu Bhavanai (400mg/kg)

## **Results of Anti- ulcer (Hyperacidity) activity of *Indhuppu bhavanai* (IB) in wistar albino rats**

### **Gastric volume**

The gastric volume was increased in aspirin induced group when compared to control group. Administration of ranitidine and IB showed a significant ( $p<0.01$ ) decrease in gastric volume level, when compared to negative control.

pH.

The pH level was decreased ( $p<0.01$ ) in the aspirin induced method, when compared to control group. Administration of Ranitidine and IB showed a significant ( $p<0.01$ ) increase in pH level, when compared to negative group animals. Results were showing Table 24.

### **Free and total acidity**

The free acidity (mEq/L/100g) was increased ( $p<0.01$ ) in the aspirin induced animals, when compared to control group. Administration of IB and ranitidine showed a significant ( $p<0.01$ ) decrease in free acidity, when compared to negative control.

Total acidity (mEq/L/100g) was increased ( $p<0.01$ ) in aspirin induced model, when compared to control group. Administration of ranitidine and IB showed a significant ( $p<0.01$ ) decrease in total acidity, when compared to negative control.

### **Conclusion**

*Indhuppu bhavanai* exhibited anti ulcer activity in aspirin induced ulcer model for screening anti ulcer drugs. The percentage of inhibition of ulcer was 91.5%, 54.5%, 75.5% produced by the treatment of standard drug Ranitidine, *Indhuppu bhavanai* (IB) at the dose level of 200mg/kg and 400mg/kg respectively. In case of vehicle control, aspirin induced rats showed increase in acid secretion, which in turn caused increase in gastric volume, low pH, increased free and total acidity resulting in increase of ulcer index.

*Indhuppu bhavanai* (IB) when administered at dose level of ( 200mg/kg and 400 mg/kg) produced a reduction in the gastric fluid volume, free acidity, total acidity and ulcer index significantly in comparison with control group.

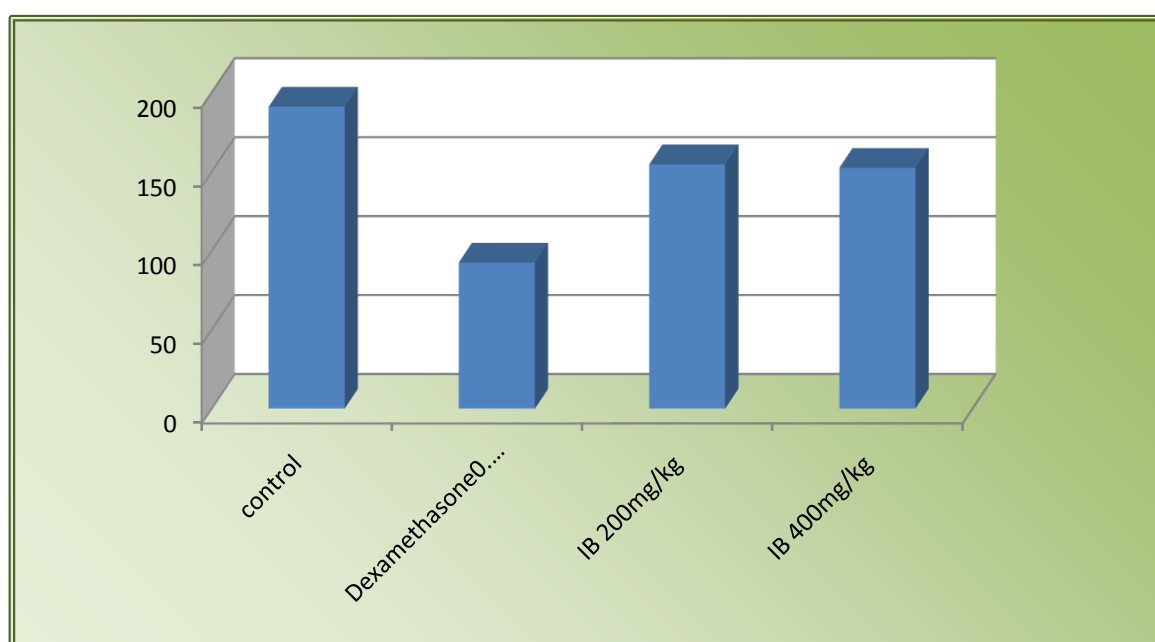
Hence, based on the results, it can be concluded that the *Indhuppu bhavanai*, significantly  $p<0.05$  and  $p<0.01$ , decreased the ulceration in Aspirin induced ulcer model in rats which suggest a direct ulcer protective effect on the gastric mucosa at the dose level of 200mg/kg and 400mg/kg respectively. The result was represented in table 26.

**Table 27 : Effect of *Indhuppu bhavanaion* cotton pellet induced granuloma model**

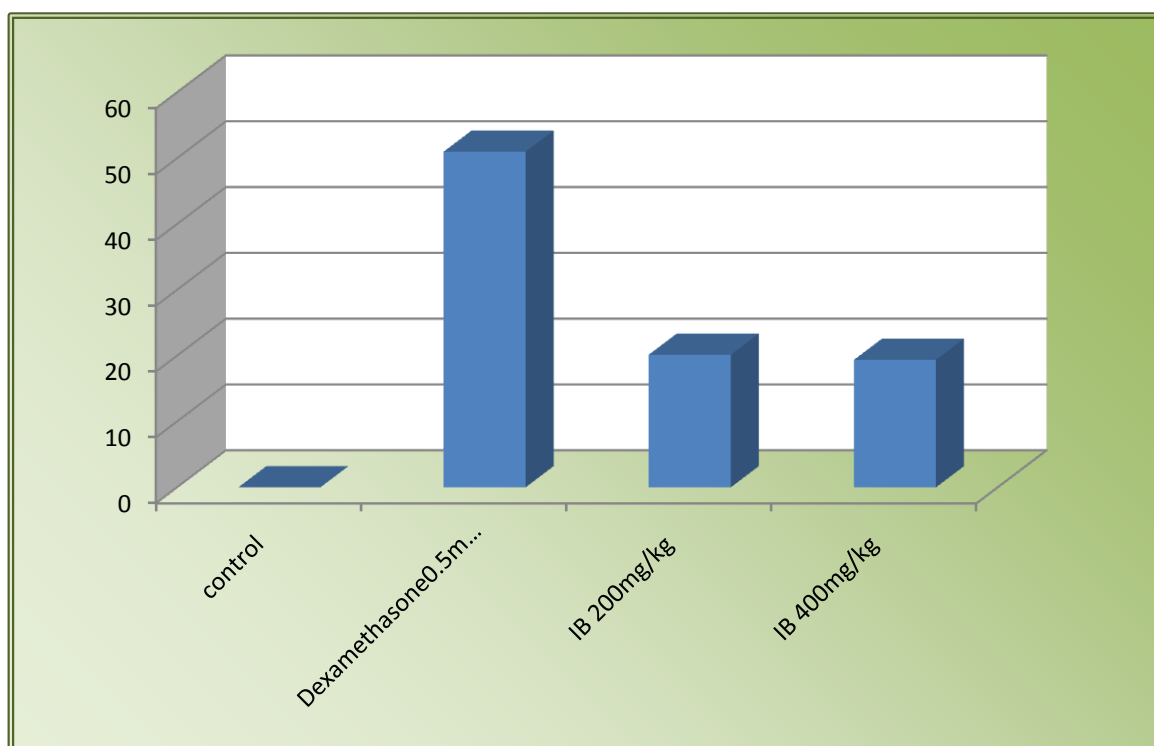
Groups	Treatment	Mean wet weight of pellet(mg)	Percentage inhibition	Mean dry weight of pellet(mg)	Percentage inhibition
I	Control	191.16±1.60	0	40.60±0.12	0
II	Dexamethasone(0.5 mg/kg)	92.5±3.39*	51	22.4±1.64*	44.2
III	Indhuppu bhavanai(200mg/kg)	154.66±3.78	20.15	35.4±0.26*	11.94
IV	Indhuppu bhavanai(400mg/kg)	152.50±3.45*	19.37	30.2±1.20*	24.87

N= 6, values are expressed as mean± SEM P<0.05 when compared with control. The results were analyzed by ANOVA followed by Dunnet's test (P value less than 0.05 was considered as statistically significant).

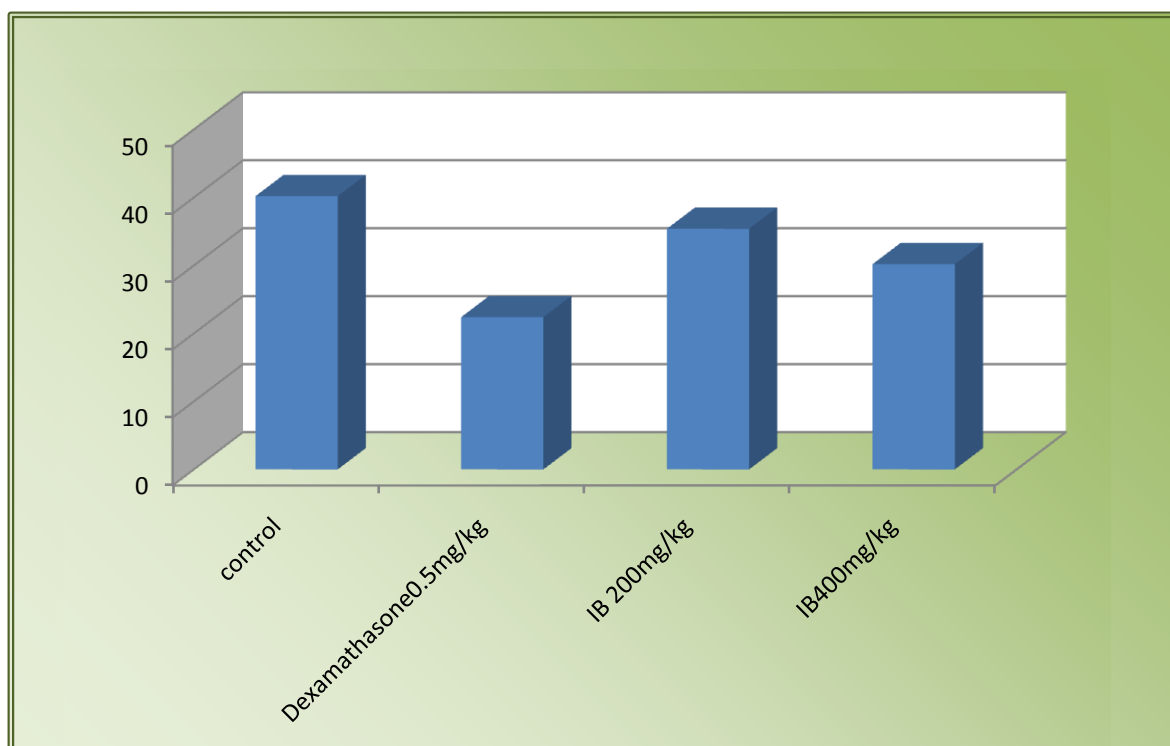
**Table 25 :Effect of *Indhuppu bhavanaion* the mean wet of cotton pellet induced granulomamodel**



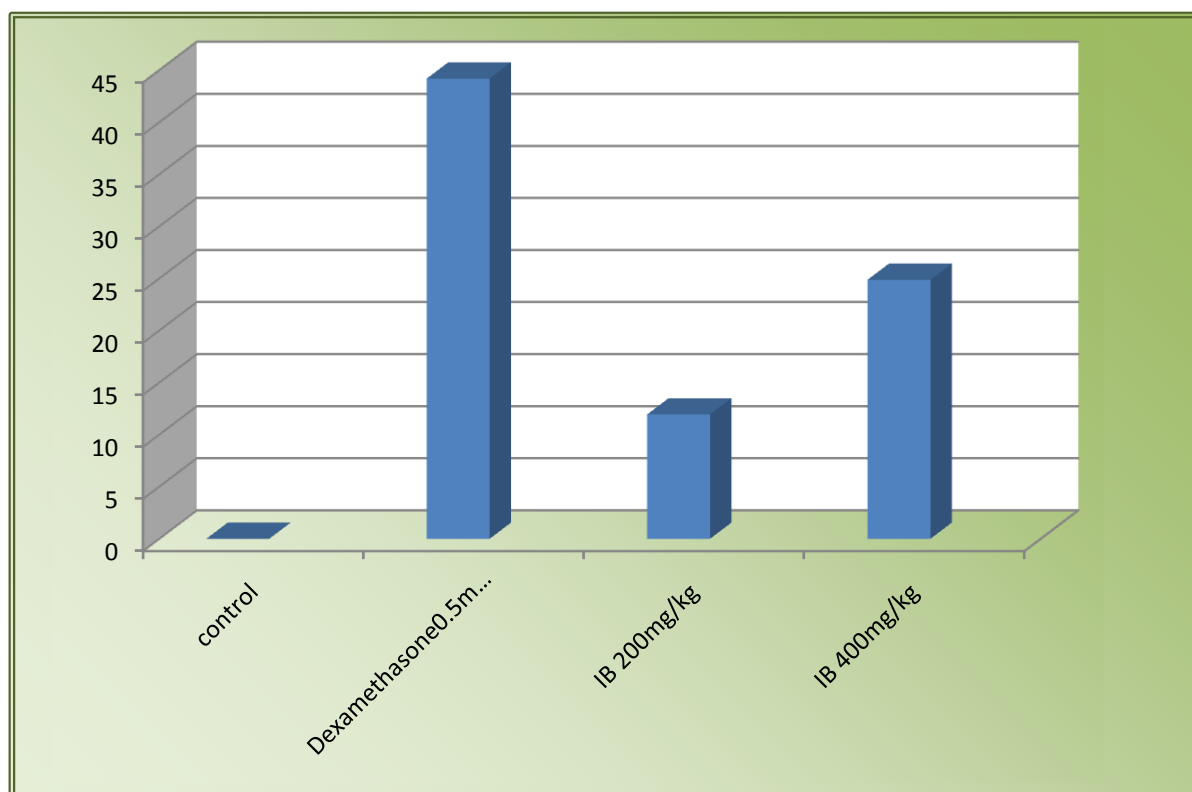
**Table 26 :Effect of *Indhuppu bhavanaion* percentage inhibition on wet cotton pellet induced granuloma model**



**Table 27 :Effect of *Indhuppu bhavanaion* mean dry of cotton pellet induced granuloma model**



**Table 28 :Effect of *Indhuppu bhavanaion* percentage inhibition on dry cotton pellet induced granuloma model**



## RESULTS

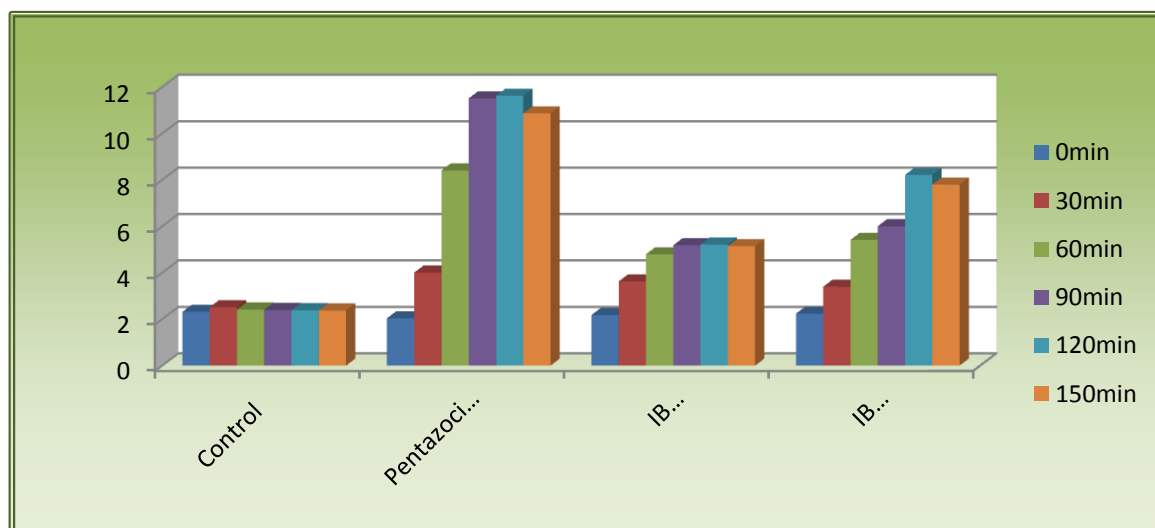
The results indicate that *Indhuppu bhavanai* at the dose level of 200mg/kg and 400mg/kg produced a decrease in wet granuloma weight  $154.66 \pm 3.78$  (20.15% inhibition) and  $152.50 \pm 3.45^*$  (19.37% inhibition) respectively when compared to control. Similarly there was a significant decrease in dry granuloma weight  $35.4 \pm 0.26^*$  (11.94% inhibition) and  $30.2 \pm 1.20^*$  (24.87% inhibition) respectively compared to control. Among the two doses 400 mg/kg showed slightly lower reduced weight of granuloma than standard drug which was showed in table 27. Thus it was concluded that administration of *Indhuppu bhavanai* at the dose of 400 mg/kg exhibited significant ( $p < 0.05$ ) anti-inflammatory activity in Cotton pellet granuloma model of inflammation in rats.

**Table28 :Pharmacological analysis – Analgesic activity of *Indhuppu bhavanai* in Swiss albinomice**

Groups	Treatment	Reaction time in sec					
		0min	30min	60min	90min	120min	150min
I	Control	2.32±0.11	2.52±0.03	2.42±0.04	2.40±0.01	2.39±0.01	2.38±0.04
II	Pentazocine (5mg/kg)	2.04±0.18	4.01±0.81	8.41±0.36	11.52±0.37**	11.63±0.39**	10.87±0.63*
III	Low dose(200mg/kg)	2.18±0.46	3.63±0.36	4.80±1.11	4.80±1.11*	5.22±0.24	5.15±0.42
IV	High dose 400mg/kg).	2.24±0.21	3.40±0.77	5.42±0.85	6.01±1.13* *	8.22±0.24* *	7.80±0.26

N= 6, values are expressed as mean± SEM P<0.05 ,P<0.01 when compared with control. The results were analyzed by ANOVA followed by Dunnet's test (P value less than 0.01 was considered as statistically significant)

**Table 29 :Analgesic activity of *Indhuppu bhavanai* by Eddy's hot plate method**



### **Result of Analgesic activity of *Indhuppu bhavanai* in swiss albino mice**

Analgesic activity was carried out by Eddy's Hot plate method. *Indhuppu bhavanai* at the two doses 200 mg/kg showed significant ( $p < 0.05$ ) analgesic activity at reaction time 90 min ( $4.80 \pm 1.11^*$ ) and 400 mg/kg showed significant ( $p < 0.01$ ) analgesic activity at 120 min ( $8.22 \pm 0.24^{**}$ ) was slightly lower than the standard drug Pentazocine  $11.63 \pm 0.39^{**}$ .

From these results it was obvious that *Indhuppu bhavanai* has significant analgesic activity.

# DISCUSSION



## 9. Discussion

The drug *Indhuppu bhavanai* was selected from the Siddha literature “*Kadukkai vallaraiin thani maanbu*” to validate the safety and its pharmacological activities (Anti ulcer, anti-inflammatory and analgesic) in animal models.

The ingredients of the test drug was identified and authenticated by Siddha experts. The drug was prepared as per the procedure and subjected to various studies such as qualitative, quantitative, toxicity and pharmacological activities. Qualitative analysis includes Chemical analysis and Physicochemical properties of *Indhuppu bhavanai*.

Quantitative analysis included ICP-OES and HR SEM analysis to reveal its potency and effectiveness against the disease.

From the above analysis we came to know the presence of active ingredients responsible for its activity.

### **Literary collections:**

Literary collections include drug review, which consist of both Botanical aspect, Gunapadam aspect, Pharmacological review that supported the study.

### **Chemical analysis:**

Chemical analysis of the drug *Indhuppu bhavanai* revealed the presence of Iron, Sulphate, Chloride, Calcium, tannic acid and alkaloid.

### **Sulphate :**

Sulphate gives strength to the mucous membrane of the stomach, intestine and also promote the appetite.

### **Chloride :**

It regulates the water balance by maintaining the osmotic pressure of the body fluids and helps in the formation of Hcl in gastric juice which reduces the gastric problems.

**Calcium :**

Calcium in the form of calcium carbonate is an antacid. It is an absorbable antacid, which neutralizes the gastric acid medium.

**Tannic acid:**

The properties of tannic acid was hardening the mucous membrane to exert a protective effect and it has anti- ulcer (Hyperacidity) property.

These chemical elements present in *Indhuppu bhavanai* enhances the anti -ulcer activity of the drug.

**Physico chemical analysis**

In physico chemical analysis, the pH of *Indhuppu bhavanai* was found to be in the range of 3.91. This shows the acidic nature of the medicine. Thus it can be easily absorbed from the gastrointestinal tract on oral administration.

The acid insoluble ash value denotes the drug quality. The drug possess with in normal value (0.1%) of acid insoluble ash indicating that the preparation did not contain any sand, dust and stones.

The loss on drying value of *Indhuppu bhavanai* was found to be 6.01% w/w, hence the drug will not lose much of its volume on exposure to the atmospheric air at room temperature.

The microbial load analysis confirms *Indhuppu bhavanai* was free from microbial organisms and fungal infections.

In ICP-OES study, heavy metals were found below detection limit in *Indhuppu bhavanai*. Calcium, Potassium, Sodium, Phosphorous were present.

In HR SEM analysis, the particle size of *Indhuppu bhavanai* showed irregular morphology and distributed in near nano range. *Indhuppu bhavanai* has particle size of 0.06 to 0.15 $\mu$ . This ensures the absorption of the drug was more active and the drug have increased bio-availability.

### **Toxicological studies:**

In acute oral toxicity study, there were no abnormal signs and behavioral changes in rats upto the dose level of 2000 mg/kg body weight administered orally. No mortality was observed in all groups. All the vital organs were normal. In Repeated dose 28 day oral toxicity and Repeated dose 90 day oral toxicity study, the experimental animals were sacrificed by excessive anesthesia and blood samples were collected and sent for investigation. There were no significant changes in body weight, food and water intake, hematological parameters, renal parameters, Liver function test, Lipid profile and blood glucose level. The organs were collected and sent for histopathology study. It revealed the organs such as heart, kidney, liver, spleen and stomach was normal in Control, Mid dose and High dose. Thus the toxicological study of the test drug greatly establishes the safety and gives the justification for long time administration.

### **Pharmacological studies:**

The pharmacological study was carried out in the animal model Wistar albino rats and mice. Three activities were seen in the drug *Indhuppu bhavanai*. The activities were

Anti -ulcer (Hyperacidity)

Anti -inflammatory

Analgesic

#### **Anti- Ulcer activity (Hyperacidity)**

*Indhuppu bhavanai* (IB) when administered at dose level of ( 200mg/kg and 400 mg/kg) produced a reduction in the gastric fluid volume, free acidity, total acidity and ulcer index significantly in comparison with control group. Hence, based on the results, it can be concluded that the *Indhuppu bhavanai*, significantly decreased the ulceration in Aspirin induced ulcer in rats which suggest a direct ulcer protective effect on the gastric mucosa at the dose level of 200mg/kg and 400mg/kg.

#### **Analgesic activity**

Analgesic activity was carried out by Eddy's Hot plate method. Analgesic effect lasted for a period of 120 min was found to be possess significant ( $p < 0.01$ ) analgesic activity at the dose level of 400mg/kg by increase in reaction time (Increase threshold potential of pain). From these results it was obvious that *Indhuppu bhavanai* has significant analgesic activity.

**Anti inflammatory activity**

Administration of Indhuppu bhavanai at the dose of 200 mg/kg and 400 mg/kg exhibited significant  $p < 0.05$  anti inflammatory activity in Cotton pellet granuloma model of inflammation in rats.

From the discussion, it was concluded that the test drug *Indhuppu bhavanai* was a safe and a potent drug for *Pitha Gunmam* (Hyperacidity).

# SUMMARY

## 10. SUMMARY

- The test drug *Indhuppu bhavanai* was selected from the siddha literature “*Kadukai vallaraiin thani maanbu*” for its anti-ulcer (Hyperacidity), anti-inflammatory and analgesic activities.
- The test drug was prepared by the given procedure. All the ingredients were identified and authenticated by the experts.
- Review of literature in various categories were carried out. Siddha aspect, botanical aspect and mineralogical aspect disclosed about the drug and the disease
- The drug was subjected to analysis such as physicochemical, phytochemical, chemical and also instrumental analysis which provided the key ingredients present in the drug thus it accounts the efficacy of the drug
- Toxicological study was made according to OECD guidelines comprising acute, sub-acute and sub chronic toxicity study. It screens the safety of the drug which attributes its utility in long time administration..
- Pharmacological study revealed that the drug *Indhuppu bhavanai* exhibited significant anti –ulcer (Hyperacidity), anti inflammatory and analgesic activity in animal models.
- From the results and the statistical analysis it was proved that the drug *Indhuppu bhavanai* has,
  1. Anti-ulcer
  2. Anti inflammatory
  3. Analgesic activity
  4. No side effect
  5. No adverse effect

Hence the trial drug *Indhuppu bhavanai* was safe for clinical trial.

# CONCLUSION

## 11.CONCLUSION

From the literature evidence, Physico chemical analysis, chemical analysis, Toxicological evaluation and Pharmacological studies, the drug *Indhuppu bhavanai* have anti-ulcer, anti-inflammtory and analgesic activity. It was concluded that the *Indhuppu bhavanai* can be used in the management of *Pitha gunmam*( Hyperacidity)



# ANNEXURE

## 12. ANNEXURE



### The Tamil Nadu Dr. M.G.R. Medical University

#69, Anna salai, Guindy, Chennai-600 032.

This certificate is awarded to

Dr./Mr./Ms. C. RUBIKA DEVI.....

for participating as Resource Person / Delegate in the Fourteenth Workshop on

### “Research Methodology & Biostatistics”

for AYUSH Post Graduates & Researchers

Organised by the Department of Siddha

The Tamil Nadu Dr. M.G.R. Medical University from 5th to 9th May 2014.

Dr. N. KABILAN M.D. (Siddha)  
Reader, Dept. of Siddha

Dr. JHANSI CHARES, M.D.  
Registrar

Prof. Dr. D. SHANTHARAM, M.D., D.Diab.,  
Vice-Chancellor



# K.K. COLLEGE OF PHARMACY

(Approved by AICTE, PCI & Government of Tamilnadu and  
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Ref: 4533/KKCP/2015

Date: 10.08.2015

## APPROVAL CERTIFICATE

This is to certify that the project titled "*Safety and pharmacological profile of Indhuppu*

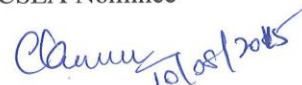
*Bhavanai*" has been approved by IAEC and the details are furnished under

Project Code	Name of the species	Breakup sexwise	Weight	Number proposed	Number approved
KKCP/2015/037	Wistar Albino rat	40 male + 46 female	150-200gms	90	86
	Swiss albino Mice	12 male + 12 female	20 - 25 gms	24	24
Wistar Albino rat – Eight six only ; Swiss albino Mice – Twenty four only					


Chairman IAEC

  
(Prof. A. Meena)




CPCSEA Nominee

  
(Dr. C. Kathirvelan)

  
Veterinary Officer

  
(V. VAIDHAYANATHAN)

Members

  
Dr. K. Sadasivan Pillai  
  


# CERTIFICATE

This is certify that the project title... SAFETY PROFILE OF -  
"INDHUPPU BHAYANAI" (12 Male + 12 Female Kustar Abinorato)  
 has been approved by the IAEC. (No: NIS/IAEC-I/2016/05)

Name of Chairman/Member Secretary IAEC:  
 nominee:

Dr. B. R. SENTHIL KUMAR

Signature with date

Chairman/Member Secretary of IAEC:

[Signature]  
 24, Feb 2016

Name of CPCSEA

K. NACHMURU

[Signature]  
 17/2/2016

CPCSEA nominee:

(Kindly make sure that minutes of the meeting duly signed by all the participants are maintained by Office)



**NATIONAL INSTITUTE OF SIDDHA, CHENNAI - 600047**

**CERTIFICATE OF BOTANICAL AUTHENTICITY**

Certified that the following plant drugs used in the Siddha formulation "**Indhuppu Bhavanai**" (Internal) taken up for Post Graduation Dissertation studies by **Dr.C.Rubika Devi**, M.D.(S), II year, Department of Gunapadam, 2015, are identified and authenticated through Visual inspection, Experience, Education & Training, Organoleptic characters, Morphology, Micromorphology and Taxonomical methods as

*Centella asiatica* (Apiaceae), Whole plant

*Citrus limon* (Linn.) Burm. f. (Rutaceae), Fruit

*Zingiber officinale* Rosc. (Zingiberaceae), Rhizome

*Phyllanthus emblica* Linn. (Euphorbiaceae), Fruit



Certificate No: NISMB1862015

Date: 14-8-2015

Authorized Signatory

**Dr. D. ARAVIND**, M.D.(s), M.Sc.,

Assistant Professor

~~Department of Medicinal Botany~~

National Institute of Siddha  
Chennai - 600 047, INDIA

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15.3.2016

**CERTIFICATE**

Certified that the samples submitted for identification by Dr. C. Rubika Devi, III year MD Student, Department of Gunapadam, National Institute of Siddha, Sanatorium, Chennai-600 047 is identified as Indhuppu – Sodium chloride (Impura)



(R. Shakila )  
Research Officer (Chemistry)

*P. Elankani*  
(Dr. P. Elankani) 15/02/16

For Research Officer (Scientist 2)-I/c





**SOPHISTICATED ANALYTICAL INSTRUMENT FACILITY**  
**INDIAN INSTITUTE OF TECHNOLOGY, MADRAS**  
Chennai - 600 036, INDIA

### CERTIFICATE

This is to certify that Herbal/Mineral Drug **Indhuppu Bhavanai** formulated by **Dr.C.Rubikadevi**, III year M.D(S), Department of GUNAPADAM, National Institute of Siddha, Chennai-47. Was analysed (qualitative/quantitative) by, SEM and ICPOES methods at SAIF, IITM, Chennai-36, during March 2016.

[DR.R.MURUGESAN]



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